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MARCH, 1951

ORIGINAL ARTICLES

THE PROGNOSIS IN ARTERIAL HYPERTENSION:

Report on 117 Patients under 53 Years of Age Followed 8 to 10 Years*

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In deciding whether drastic dietary or surgical treatment is indicated for a given patient with established hypertension, it is essential for the physician to know something of the natural course of the disease and of the signs that indicate an ominous prognosis. In attempting to provide an answer to this question, the records of 119 consecutive patients with "essential hypertension" examined at the University Hospital in the years 1938 to 1940 were selected. These individuals were all carefully studied, had blood pressures exceeding 160/110 on 2 or more determinations, were under the age of 53, and did not have primary renal disease or terminal complications such as coronary thrombosis, recent cerebrovascular accidents, congestive failure or severe renal impairment as manifested by an elevated nonprotein nitrogen or markedly diminished urea clearance. In short, all of these patients would have been considered suitable candidates for splanchnicectomy according to our present indications, and 20 of them were in fact accepted for surgery but did not return for the operation for various reasons. The status of 117 of these patients was determined 8 to 10 years later by letter to the patient's physician or by reexamination at the University Hospital in the case of 72% of the survivors.

Results. The 117 patients on whom follow-up data could be obtained have

* These studies were supported by a grant from the National Institutes of Health, United States Public Health Service.

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been studied from 3 standpoints: 1) gross mortality rate and type of death; 2) factors influencing prognosis; and 3) status of the survivors.

1. MORTALITY RATE AND TYPE OF DEATH. Of the original 117 patients, 46% died from hypertensive complications, 2% from unknown causes, 6% for reasons unrelated to high blood pressure, and 46% were still alive at the end of the 10-year interval (Table 1).

TABLE 1.—MORTALITY RATES AND CAUSE OF DEATH IN HYPERTENSION*

			Reported cause of death					
Sex	*Number of cases*	*Dead*	*Cardiac*	*Renal*	*Cerebrovascular accident*	*Cardiovascular renal*	*Non-hypertensive*	*Cause unknown*
Females	73	32 (43.8%)	9 (12.3%)	2 (2.7%)	13 (17.8%)	3 (4.1%)	4 (5.5%)	1 (1.4%)
Males	44	31 (70.4%)	9 (20.4%)	1 (2.3%)	13 (29.5%)	4 (9.1%)	3 (6.8%)	1 (2.3%)
TOTAL	117	63 53.8%	18 15.4%	3 2.5%	26 22.2%	7 6.0%	7 6.0%	2 1.7%

* All patients were under the age of 53 and did not have elevation of the nonprotein nitrogen, recent cerebrovascular accident, or congestive failure at start of the interval. They were followed up 7 to 11 years after the initial examination, data on 90% being obtained within an 8 to 10 year period. Cardiovascular renal complications was used to denote deaths due to hypertension in which no system could be said to have failed more strikingly than any other.

The hypertensive deaths were about equally divided between cardiac and cerebrovascular causes. Uremic deaths were uncommon, showing that the average patient with "essential hypertension" and fair kidney function is unlikely to succumb to renal failure.

2. FACTORS INFLUENCING PROGNOSIS. (a) SEX. From Table 1 it is evident that the prognosis is poorer for males than females. The type of hypertensive death was the same in each sex and there was no significant difference in the age distribution, range of blood pressure, fundus grading, or frequency of vascular complications. Females, therefore, with a comparable severity of hypertensive disease outlived males in a ratio of about 3:2.

(b) EFFECT OF INITIAL HYPERTENSIVE COMPLICATIONS ON MORTALITY. The finding on the initial examination of electrocardiographic abnormalities, cardiac enlargement, or a history of a cerebrovascular accident or focal encephalopathy, was considered evidence of "hypertensive complications."* *Table 2* illustrates the marked prognostic importance of such manifestations. For example, at an initial diastolic blood pressure level of 120 to 129, 2 out of 13 patients with such complications survived, whereas 12 out of 14 patients with uncomplicated hypertension lived through the interval. Complications of hypertension similarly affected the outcome at every blood pressure level studied. This increased death rate was not the result of an increased number of males with complicated hypertensive disease. A detailed analysis of the effect of the type of complication upon the prognosis follows in a later section of this report.

(c) EFFECT OF INITIAL BLOOD PRESSURE LEVELS ON THE PROGNOSIS. Table 2 indicates that the blood pressure *per se* had little effect on the outcome. Of 22 patients with initial diastolic blood

* Electrocardiographic abnormalities usually consisted of inverted T waves in leads I and II, or significant left axis deviation as interpreted through the courtesy of Dr. Frank N. Wilson; cardiac enlargement was defined as an increase of more than 15% above the predicted normal based on height and weight according to the standards of Hodges and Eyster[6].

pressure between 110 and 119 but without hypertensive complications, 77% survived; whereas 59% of 17 similar patients with diastolic pressures over 130 lived through the 9-year interval. It will be seen that patients with higher blood pressure levels tended to have hypertensive complications more frequently, and therefore as a group they had a worse prognosis. On the other hand, when the effect of such complications is excluded, it is by no means certain that blood pressure levels bear any important relation to prognosis.

(d) EFFECT OF AGE AND DURATION OF HYPERTENSION ON MORTALITY. Since survival might be the result of duration of hypertension at the time first seen, the data were studied in this regard. The age of the patient at the time of the initial examination had only a slight effect on the mortality when such variables as initial height of blood pressure and presence of vascular complications were accounted for. Thus, of patients under 40 with uncomplicated hypertension, 14 survived and 3 died, while in those over 40, 23 survived and 11 died. Young persons exhibiting signs of hypertensive complications had a severe mortality rate: 3 lived and 14 died, while of those over 40 in the same group, 10 survived and 25 died. This young group did not have a high proportion of males nor high blood pressure levels, hence the presence of the complications of hypertension, especially in a young person, carried a serious prognostic implication. However, with this exception, it did not appear that age *per se* was of any great importance in affecting the outlook in essential hypertension.

The known duration of hypertension prior to the first examination as obtained from the history did not appear to influence the survival rate. The median duration of hypertension prior to the initial examination was 4 years in the survivors and 3 years in those who died, but the differences were not significant. Most patients knew of their hypertension only within the year of the initial examination: it is probable that it had been present for a longer period but passed unrecognized. Therefore the data on this point are probably unreliable, but it does not suggest that patients in our series dying of hypertension happened to be seen later in the course of their disease than the survivors. It was apparent that the duration of the disease was in no way related to prognosis, since of 10 persons who were known to have had elevations of blood pressure for 15 to 20 years prior to the first examination, 5 survived an additional 9 years, and 5 died. This is approximately the mortality rate of the series as a whole.

TABLE 2.—EFFECT OF INITIAL BLOOD PRESSURE AND OF CARDIAC OR CEREBROVASCULAR COMPLICATIONS ON PROGNOSIS IN HYPERTENSION

Initial diastolic blood pressure mm. Hg	*Complications at initial examination**			
	Present		*Absent*	
	No. living	*No. dead*	*No. living*	*No. dead*
110–119	3	2	17	5
120–129	2	11	12	2
130–139	3	9	6	5
140–170	3	17	4	2
TOTAL	11	39	39	14

* Complications included prior cerebrovascular accident (6 cases), electrocardiographic abnormalities, or heart size increased by more than 15% of normal. In this and succeeding tables, total number of patients is slightly less than the original 117 owing to exclusion of 9 patients who died of nonhypertensive or unknown causes and of a few patients in whom adequate data were not present on initial examination.

(e) EFFECT OF CARDIAC COMPLICATIONS ON THE MORTALITY. All 16 patients with cardiac enlargement alone or in conjunction with electrocardiographic changes died, 6 within 2 years of the initial examination. The cardiac enlargement was not more common in the male sex but did tend to occur at higher initial levels of blood pressure. However, even taking into account this fact, it is readily apparent that the enlargement added greatly to the mortality rate. It is noteworthy that many of these patients with cardiac enlargement did not complain of marked dyspnea nor signs of congestive failure at the time of the initial examination.

Of 39 patients with abnormal electrocardiograms alone, 25 failed to survive the interval. This represents a slight increase over the gross mortality rate of the series as a whole, but indicates that the electrocardiographic abnormality was not as serious a prognostic sign as was cardiac enlargement.

It was somewhat unexpected to find that in these individuals the type of death was as likely to be cerebrovascular as cardiac. Four of the cardiac deaths were ascribed to coronary thrombosis, and occurred 3 to 9 years later. The initial electrocardiogram showed left axis deviation in 2 instances and T-wave inversion only in 2 instances. Four patients complained of angina pectoris on initial examination: 1 is still living, 2 have died of a cerebrovascular accident, and 1 died of coronary thrombosis.

(f) EFFECT OF CENTRAL NERVOUS SYSTEM SYMPTOMS AND COMPLICATIONS ON OUTCOME. Cerebrovascular complications in hypertension are notably difficult to predict. Seventy-five patients who complained of headaches or dizziness at the initial examination had essentially the same mortality rate and type of death as 24 patients without such symptoms. It was also found that the frequency of cerebrovascular death did not increase with higher initial blood pressure levels, but did tend to occur more frequently in the older patients. Advanced retinal vascular disease might be presumed to reflect concomitant cerebrovascular lesions. This was not found to be true: of 20 patients with grade 1 or 2 fundi at the start of the interval, 55% died of cerebral complications, whereas of 25 patients with grade 3 fundi, 60% died of cerebrovascular disease.

Twelve patients died of a cerebrovascular accident within 2 years of the initial examination. Five had no complaint of headache when they were first seen. Only 1 of the 12 had had a prior cerebrovascular accident, and 4 had diastolic blood pressures below 130 mm. of mercury. It will be seen that even when cerebrovascular death was relatively imminent, neither headache nor marked elevation of the blood pressure was particularly helpful in predicting the outcome.

Six patients had one or more transient cerebrovascular accidents before the first examination. In 3 of them, neurologic residua were present at the time they were first seen. Four of these patients died of subsequent strokes after surprisingly long intervals: 6, 8, 9, and 13 years from the date of the initial accident. Two survived the 9-year interval, living 11 and 13 years after having sustained a transient hemiplegia of several months' duration. Both were without cerebrovascular symptoms at the time of final examination, although in neither case had there been any reduction in blood pressure during the interval.

(g) EFFECT OF RETINAL VASCULAR DISEASE ON THE MORTALITY. Fundus grading has been used extensively in estimating prognosis in hypertension[9]. An attempt, therefore, was made to examine the influence of the fundus classification on the outcome. Tabulation of fundus grading in relation to diastolic blood pressure levels and rate of hypertensive complications revealed

such a close correlation between the two that little opportunity occurred to compare the value of different fundus gradings upon the prognosis in patients with comparable blood pressures. The 4 patients with grade 4 changes included in this series all had a fatal outcome within 2 years of the initial examination. As has been mentioned previously, the degree of retinopathy is not of value in predicting the type of death that will ultimately occur; but as a direct reflection of the vascular degeneration accompanying the hypertensive disease process, it is undoubtedly a useful guide to the future.

(h) EFFECT OF RENAL STATUS ON THE MORTALITY RATE. Three of 4 patients with urea clearance below 50% died; 7 of 11 individuals with 50 to 75% of normal function died. Even more ominous was the consistent presence of albuminuria. Although this manifestation occurred more frequently in patients with high initial diastolic pressures, or cardiovascular complications, the data would suggest that in patients with these changes the added presence of persistent albuminuria made the prognosis definitely worse.

(i) EFFECT OF CHANGES OF BLOOD PRESSURE DURING THE INTERVAL ON THE MORTALITY. In 24 of the 54 patients who died hypertensive deaths, the diastolic blood pressure 1 week to 2 years before death was known (solid dots in Fig. 1). It will be seen that in 2 cases it had increased more than 20 mm. from the levels at the initial examination, in 4 cases it had fallen more than 20 mm., and in 18 it had remained within these limits. There was a general tendency for the blood pressure to fall slightly in cases with cerebral deaths, and to rise in patients with cardiac deaths. The significance of these findings, whether the cause or the result of the terminal illness, is unknown, since the data obtained by correspondence are not entirely dependable and no blood pressure information at all could be obtained in more than half of the patients who died. However, there is certainly no evidence that an increase in blood pressure is a common event just prior to death in hypertension, nor that a rising blood pressure predisposes to the development of a cerebrovascular accident.

3. STATUS OF THE SURVIVING PATIENTS. From personal interview or questionnaire it was learned that of 51 living patients, 19% are symptom-free at present, and 61% have mild symptoms referable to hypertension, but are useful citizens capable of doing their daily work. Adding these 2 groups together, one finds that 80% of the living patients (35% of the entire series) have remained capable and useful people without severe distress. Forty-three percent of the females and 13% of the males in the entire series fall into this category.

Further analysis of the symptoms reveals that the hypertensive complaints of headaches and dyspnea if initially present had rarely improved, while if they were absent at the first examination they have developed in the interval in an appreciable number of patients. In most cases, however, the symptoms in the survivor were not disabling. Furthermore, many of the patients without retinal or cardiovascular renal changes initially have now acquired evidence of such damage, whereas those who were previously abnormal have shown little tendency toward improvement.

Changes in the blood pressure in the survivors were of considerable interest. The open circles in the graphic record (Fig. 1) reveal the variation between initial and final readings. The diastolic blood pressure remained within ± 20 mm. of the original value in 38 of the 43 surviving patients in whom satisfactory data were obtained. Five patients with diastolic blood pressures exceeding 140 mm. on the initial examination are still living, and 3 of them

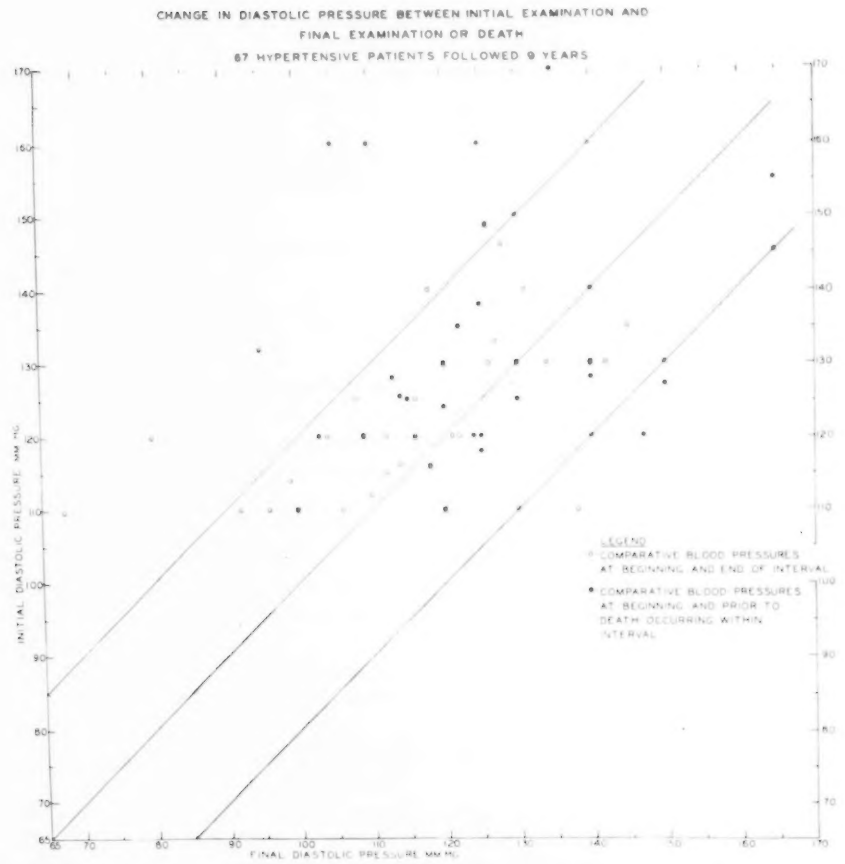
CHANGE IN DIASTOLIC PRESSURE BETWEEN INITIAL EXAMINATION AND FINAL EXAMINATION OR DEATH
67 HYPERTENSIVE PATIENTS FOLLOWED 9 YEARS
INITIAL DIASTOLIC PRESSURE MM HG
FINAL DIASTOLIC PRESSURE MM HG
LEGEND
COMPARATIVE BLOOD PRESSURES AT BEGINNING AND END OF INTERVAL
COMPARATIVE BLOOD PRESSURES AT BEGINNING AND PRIOR TO DEATH OCCURRING WITHIN INTERVAL

FIG. 1. Change in diastolic blood pressure during follow-up observation. Graph is so constructed that points on the middle diagonal line represent no change in diastolic blood pressure during the interval, while points above the upper diagonal line indicate decrease of more than 20 mm. of mercury. It will be seen that changes in blood pressure in both survivors and fatalities rarely exceeded the limits of ± 20 mm. of mercury diastolic pressure.

still have blood pressures above 130 diastolic, 9 years later. All were females initially between the ages of 41 and 47, and, except for electrocardiographic abnormalities in 2, no complications of hypertension were present initially. All 3 were classified as without serious disabling symptoms at the time of the follow-up examination, but only 1 was entirely asymptomatic.

Reductions in diastolic blood pressure exceeding 20 mm. have occurred in 4 of the survivors. In 2 cases there was a decline to levels of 95 and 118 mm. of mercury respectively. In 2 patients the blood pressure fell to normal. One apparently had a significant improvement in the blood pressure but suffered a "stroke" during the interval. Her history is as follows: She was a 45-year old white female with a blood pressure of 230/140 when she was first seen at the University Hospital. She did not return for re-examination but follow-up letters disclosed that her pressure is now normal and her condition is "better than it was 10 years ago." She had a "stroke" about 4 years ago.

The second patient (Fig. 2), after

maintaining a prolonged but labile hypertension without evidence of vascular changes, has had a gradual reversion to normal blood pressure and has remained normotensive for a number of years. The details of her history are summarized in the accompanying illustration.

Discussion. Forty-six percent of the patients died within 9 years of the complications of hypertensive disease. selves are of serious prognostic importance. Although the number of cases is insufficient to draw definite conclusions, we feel that these findings in approximate order of individual importance seriously affect the prognosis: 1) evidence of hypertensive complications, in particular, cardiac enlargement, persistent albuminuria, and history of encephalopathy or cerebrovascular accident; 2) height of

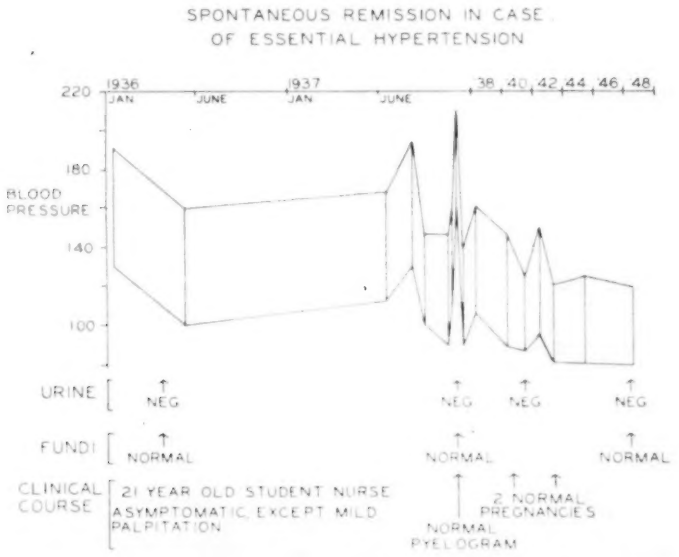

FIG. 2. Clinical observations over a 12-year period in a patient with essential hypertension, who experienced a spontaneous and sustained reduction in the blood pressure.

The expected mortality rate for the same interval of the normal U. S. population distributed by age, sex, and color in the manner of our patients was 7.8%[15]. It is thus apparent that we selected a group of patients who would have had a reasonably good life expectancy if they had not had hypertension. Our gross mortality rate of about 50% at 9 years agrees fairly well with the experience reported by others, when the widely varying criteria of selection and duration of follow-up periods are considered[10-12].

We have made a serious attempt to select those factors which in them- the diastolic blood pressure; 3) male sex; 4) abnormality of the electrocardiogram; 5) hypertensive retinopathy. Certain factors did not appear to play a significant role in increasing the mortality rate. These included age of the patient, estimated duration of hypertensive disease, frequency and severity of headaches or dizziness at the time of initial examination. It must again be emphasized that these are only tentative conclusions; it is impossible to evaluate each individual factor in a series this small.

No attempt has been made in published reports to evaluate the prognos-

tic importance of certain findings independently of the blood pressure levels and of other factors of probable importance. Palmer[11] has recently emphasized the importance of the vascular complications in determining the prognosis in hypertension. His patients without such complications had a 22% mortality rate 8 years later, while those with more definite cardiovascular, renal or cerebral involvement had a 47 to 78% mortality rate.

Bechgaard and several other authors [1,3,4,10,14] have emphasized the seriousness of electrocardiographic or roentgenologic signs of cardiac damage in evaluating the prognosis. Others[2,5,13] in a selected series of long surviving patients did not find that these abnormalities increased the mortality rate.

Relation of retinal vascular disease to the prognosis could not be independently evaluated, since degree of retinopathy paralleled height of blood pressure and complication rate. Only 4 cases of grade 4 fundi, so-called "malignant hypertension", in association with a normal nonprotein nitrogen were included. These had the fatal prognosis described by others. Only Flaxman[5] has indicated that an appreciable number of these patients may survive.

The higher the blood pressure at the initial examination, the worse was the prognosis. This is in accord with the opinion of most investigators, although Janeway[8] indicated that the height of the blood pressure was of little prognostic import, and Bechgaard suggested that particularly in females the mortality rate did not increase with higher blood pressures. In agreement with other authors, however, we have observed frequent cases, particularly in females without hypertensive complications, in which long survival with high blood pressure levels has occurred. Blood and Perera, among others, have made this the subject of special study[2].

The type of hypertensive death was equally distributed between cardiac and cerebrovascular causes. Except when a prior cerebrovascular accident suggested a cerebral death, we have been unable to predict the type of death on the basis of the initial status of the patient. A high initial blood pressure did not predispose to apoplexy in our patients as it has in the experience of others[8,10,14].

We observed occasional reductions in blood pressure in the survivors and 2 instances of marked reductions to normal levels. One patient apparently became normotensive following a cerebrovascular accident; the other was a woman with very few signs of organic vascular disease and a very labile pressure. Rasmussen and Boe[14] found 2 cases in their 100 patients studied over a 6-year interval in whom blood pressure was reduced to normal; in the great majority the blood pressure showed no essential change. Twenty-seven percent of 100 relatively mild hypertensive patients analyzed by Bechgaard had reductions of over 10 mm. of mercury in their diastolic blood pressure 4 to 5 years later, but this can hardly be described as a significant change since spontaneous variations often exceed such limits. In his series analysis reveals that only 15 cases of essential hypertension with an initial diastolic blood pressure over 110 fell to normal on follow-up examinations. This represents about 3% of the 562 patients with initial pressures within the category included in our study and corresponds rather well with our experience. Although significant spontaneous reductions do undoubtedly occur, they are very rare and it can be assumed, we believe, that once hypertension is well established the chance of spontaneous cure or definite reduction of the pressure is quite uncommon. Any treatment, surgical or medical, which accomplishes this result over an extended period on more

than 3 to 5% of patients can be said, we believe, to have had a significant effect.

The relative constancy of the blood pressure in most of the survivors was somewhat surprising, although a similar observation was made by Rasmussen and Boe when the survivors of their series were re-examined. Within limits of plus or minus 20 mm. diastolic pressure there was no significant variation during the entire period of observation. There was no tendency for patients with less severe hypertension to have progressive elevation of the blood pressure. It may be objected that a rise or fall of 20 mm. diastolic covers too wide a range. Yet studies on hypertensive patients examined in 2 clinics of the hospital within a few days of each other, or in the same clinic 10 to 15 months apart, show fluctuations of this order of magnitude[7]. Consequently we do not believe the limits are too closely defined, and even within any limit selected there is no significant upward trend, as the median change over the 9-year interval was −2 mm. of mercury. Since the majority of patients had had established hypertensive disease for one or more years before they were referred to the University Hospital, it follows that rises in pressure must have occurred before we saw them and that once the disease became established the tendency was for the pressure to be maintained at this level more or less indefinitely.

It might be objected that the blood pressure remained constant in the survivors but continued to rise in those who succumbed to the disease in the interval. While this possibility cannot be denied, fragmentary statistics from our series would indicate that there was no significant increase in blood pressure prior to death in 18 of 20 patients whose blood pressure was recorded within 2 years of the date of death. Paullin[12], who made more careful observations on his patients, reached a similar conclusion.

The greater probability is that, with rare exceptions, after hypertensive disease becomes well established, the blood pressure usually fluctuates about a more or less constant value and the mortality is thereafter determined by the location and progression of vascular disease. Such vascular changes reflected by albuminuria, advanced retinopathy, cardiac enlargement, electrocardiographic alterations or cerebral disturbances are probably the result of ischemia due to arteriolar disease. These signs of organic disease usually parallel the height of the blood pressure but are more important than the latter in determining the mortality rate.

It is therefore necessary to determine the frequency of vascular complications in any series of hypertensive patients when the long term results of treatment are to be evaluated or compared with other reported studies. In this investigation, for example, by choosing patients with little evidence of vascular disease, an 80% survival rate could have been obtained, while if one were to select the patients with the highest complication rate a 20% survival rate would have been achieved from material of comparable age, sex and blood pressure distribution.

A steadily rising blood pressure probably indicates an acceleration of the disease process with a worsening of the prognosis, whereas a significant spontaneous reduction in blood pressure is in all probability a favorable sign. Our study would indicate, however, that such definite changes are apparently unusual once hypertension has become fully established and that after the early stages of the disease have passed, the blood pressure level does not tend to rise further and death is more dependent on the presence or progression of signs of vascular damage than on the level of the blood pressure alone. Consequently, the

common practice of assuming that the outlook is good if the blood pressure fails to rise further, once hypertension has become well established, may lead to a false sense of security. Again it is emphasized that the prognosis as well as the decision concerning the form of treatment to be instituted should depend on the evidences of vascular damage rather than on the height or change in the levels of the blood pressure. A male patient with evidence of cerebral, cardiac, or renal changes carries a serious prognosis if untreated, whereas a female patient without such abnormalities is likely to survive a long time in relatively good health with no treatment at all. These factors may be of determining importance when the physician is confronted with the often difficult decision concerning the advisability of severe dietary restriction or extensive surgery in the treatment of the patient with hypertensive disease.

Summary. 1. Of 117 carefully studied hypertensive patients under the age of 53 with blood pressures initially exceeding 160/110 who were followed up 9 years later, 46% had died of the complications of hypertensive disease. Cerebrovascular accidents accounted for approximately one-half of the deaths, and cardiac and renal complications accounted for the remainder.

2. The most important factor in determining prognosis was the initial presence of vascular disease as manifested by cardiac enlargement, electrocardiographic alterations, albuminuria, or hypertensive encephalopathy. Approximately 80% of patients with such complications died, whereas only 20% of those without evidence of vascular damage failed to survie the 9-year interval. The effect of such complications on mortality rate was independent of the height of the blood pressure, sex distribution, or other known variables.

3. In addition to the above, certain findings of lesser individual prognostic value were: a) the height of the blood pressure, b) sex of the patient (mortality rate in males was higher by a ratio of 3:2), c) degree of hypertensive retinopathy. The age of the patient and duration of hypertension had little, if any, effect on the outcome.

4. It was generally impossible to predict the type of death from the findings at the initial examination.

5. The survivors, when re-examined, showed evidence of slow progression of their hypertensive disease. However, 80% were without serious or disabling symptoms. The benign course of the disease in many patients without vascular complications is emphasized.

6. The blood pressure showed little tendency to change either in the survivors or in those who died of hypertensive disease. Many patients withstood severe hypertension for many years; only 2 survivors had a fall in blood pressure to normal levels.

7. The unreliability of the height or change in the blood pressure level as a prognostic sign in hypertension is emphasized, and the importance of determining the degree and progression of vascular damage in heart, brain, kidneys, and retina is stressed. Such data must be known before mortality rates in any series of hypertensive patients can be evaluated, and this information is of vital importance in formulating a plan of treatment for a patient with "essential" hypertension.

We are indebted to Mr. James Pingell and Miss Nancy Helen Rausch for their valuable assistance in collecting the statistical data.

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THE USE OF MERCUHYDRIN IN THE RECOGNITION OF A CARDIAC FACTOR COMPLICATING BRONCHIAL ASTHMA

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It is often difficult to differentiate early left ventricular failure from allergic bronchial asthma[5]. Particularly is this so in the older age group where hypertensive and arteriosclerotic heart disease are common. The co-existence of cardiovascular disease and bronchial asthma is by no means rare and, inasmuch as dyspnea, cough and wheezing are features common to both, the problem becomes more perplexing. Recently, 6 patients over 50 years of age with long standing bronchial asthma of allergic origin were encountered in this hospital, who suddenly failed to respond to the usual anti-allergic regimen. Administration of mercuhydrin by the subcutaneous route produced marked clearing of the pulmonary signs with dramatic relief of the dyspnea in several hours. Thereafter, these patients were kept on a maintenance dose of this diuretic with considerable improvement of the asthmatic state.

As a control, a series of 20 patients with straightforward allergic bronchial asthma, under the age of 40, were treated with 2 cc. of mercuhydrin subcutaneously during an acute asthmatic attack. No beneficial effect was noted in this group.

Case Reports. Case 1. M. R., a 71-year-old white male, was admitted to the Fourth Division Allergy Clinic on February 19, 1943, complaining of severe bronchial asthma and hay fever of 10 years' duration. There was no family history of hypersensitivity. Physical examination revealed moderate dyspnea and cyanosis of the lips. The chest was emphysematous, and numerous rhonchi, wheezes and sonorous rales were audible. Cardiac dullness was enlarged to the left. The heart sounds were of fair quality, and a soft systolic murmur was heard at the apex. The rhythm was regular, and the blood pressure was 175/90. The liver and spleen were not palpable, nor was there any peripheral edema.

Roentgen examination of the chest disclosed enlargement of the heart in the transverse diameter and marked fibroid changes at the roots of both lungs. The nasal sinuses showed thickening of the antral mucosa on Roentgen-ray. The electrocardiogram demonstrated right bundle branch block.

The red blood count was 5.5 million and the hemoglobin was 16.5 gm. The white blood count was 7800 and the differential was normal. Urinalysis showed a specific gravity of 1.014, with no albumin or sugar present. Microscopic examination disclosed an occasional hyaline cast without any cellular elements.

Skin tests were strongly positive to dust, ragweed, dog epithelium, tobacco, pork, fish and cornmeal. Antral puncture and lavage yielded turbid fluid, but no organisms were obtained on culture.

Proper hyposensitization therapy and diet were successful in adequately controlling the asthmatic symptoms until November 18, 1948. At this time, there was a marked increase in the pulmonary signs and symptoms without any appreciable response to adrenalin and aminophyllin. The neck veins were not distended; the liver was slightly enlarged, and there was no evidence of edema. A cardiac factor was suspected and 2 cc. of

mercuhydrin was given subcutaneously with marked improvement in several hours. There was a loss of 4 pounds in weight noted on the following day. Thereafter, he was placed on a maintenance dose of mercuhydrin with relief of his asthmatic paroxysms.

CASE 2. H. S., a 59-year-old white male, was a patient in the Fourth Division Allergy Clinic of Bellevue Hospital since 1938. His chief complaint was bronchial asthma of 5 years' duration. His past history disclosed angina on effort, but no episodes of congestive failure. There was no family history of allergic disease. On physical examination, slight cyanosis and moderate dyspnea, with an emphysematous type of chest were noted. Cardiac dullness was enlarged to the left on percussion. There were no murmurs, the rhythm was regular, and the blood pressure was 120/68. The lungs were hyper-resonant to percussion, and wheezes, rhonchi and sonorous rales were heard throughout the chest. The liver and spleen were not palpable, and there was no peripheral edema. Clubbing of the fingers and toes was evident.

Roentgen-ray examination of the chest revealed a normal cardiac silhouette and interstitial changes and fibrosis of the lungs with blunting of the left costophrenic angle. The electrocardiogram showed an occasional premature ventricular contraction with a horizontal type of heart and a suggestive old anterior wall infarct (inverted T_3, T_4). The urine, blood count, and blood chemical studies were within normal limits. Mazzini and Wassermann tests were positive.

Intracutaneous tests were markedly positive to dust, feathers, cottonseed, dog epithelium, and many foods. Proper anti-allergic therapy was instituted and massive doses of penicillin were given him by the Neurosyphilis Department. He did very well, with only an occasional recurrence of his asthma, which responded to epinephrin inhalation or adrenalin subcutaneously. On January 12, 1949, during his usual clinic visit, he stated that for about 5 days he was in continuous asthma. Despite his many visits to the Emergency Room for intravenous aminophyllin and subcutaneous adrenalin, his pulmonary symptoms persisted.

Physical examination at this time revealed marked dyspnea, orthopnea and slight cyanosis. The heart sounds were of fair quality and the rhythm was regular. The blood pressure was 110/70. The lungs were filled with sonorous and sibilant rales, rhonchi and wheezes. The neck veins were not distended.

The liver was not palpable nor was there any peripheral edema.

He was given 2 cc. of mercuhydrin subcutaneously and intravenous aminophyllin. The next morning a 4 lb. weight loss was noted, with marked relief of the asthma. Thereafter, he was kept on a maintenance dose of mercuhydrin in addition to his regular anti-allergic regimen, with marked improvement.

CASE 3. J. D. S., a 68-year-old white male, was a patient in the Fourth Division Allergy Clinic of Bellevue Hospital since 1943 for severe bronchial asthma of 10 years' duration. There was a family history of allergy. His past history disclosed many admissions to the wards of Bellevue Hospital for status asthmaticus. It was discovered that these attacks were ushered in by infection of the bronchopulmonary tree.

Physical examination at the clinic revealed evidence of emphysema with many rhonchi and sibilant rales in the chest. The heart was enlarged to the left on percussion, and no murmurs were heard. The rhythm was regular, and the blood pressure was 170/90. The liver was not enlarged and there was no evidence of peripheral edema. Clubbing of the fingers and toes was evident.

Roentgen-ray of the chest disclosed enlargement of the transverse diameter of the heart with interstitial changes and fibrosis of the lungs. The electrocardiogram revealed left ventricular strain with an occasional premature ventricular contraction.

The urinalysis, blood count, Mazzini test for syphilis, and blood chemical studies were normal. Skin tests were positive for dust, rabbit epithelium, ragweed, egg white, chicken and spinach. Antral puncture and lavage yielded a clear fluid with few shreds, but on culture there was no growth of organisms.

The patient was treated with dust, ragweed and proper diet with control of his paroxysmal asthmatic seizures. However, on October 14, 1948, he presented himself with an acute asthmatic attack and stated that he had been to the Emergency Room of the hospital several times for intravenous aminophyllin with no relief. At this time, examination revealed marked dyspnea, orthopnea, and cyanosis. There was loud wheezing and considerable moisture in both lungs. Subcutaneous injection of 2 cc. of mercuhydrin was given and on the following day he was considerably improved. A loss of 5 pounds in weight was noted, with clearing of symptoms. He was subsequently placed on maintenance mercuhydrin therapy in addition to the usual anti-allergic regimen, with excellent control of his asthmatic state.

CASE 4. G. R., a 60-year-old male, entered the Fourth Division Allergy Clinic of Bellevue Hospital in 1943 for chronic bronchial asthma of 8 years' duration. There was a

family history of asthma, but no history of any associated allergic disorder.

Physical examination revealed moderate dyspnea with slight cyanosis. There was an increase of the anterior posterior diameter of the chest with diminished expansion of the bases. There was slight enlargement of the heart to percussion. The heart sounds were of good quality, the rhythm was regular, and no murmurs were heard. The blood pressure was 210/110. Wheezes, rhonchi, and numerous sonorous and sibilant rales were heard in both lungs. The liver and spleen were not palpable. There was no peripheral edema but clubbing of the fingers and toes was present.

The Roentgenogram of the chest showed fibroid changes in the roots of both lungs extending to the bases, and slight increase of the transverse diameter of the heart. The red blood count was 5.5 million cells per cc., with a hemoglobin of 15.1 gm. There were 7400 white blood cells per cc. with a normal differential.

Urinalysis and blood chemical studies were within normal limits. Skin tests were positive for dust, rabbit hair, dog epithelium, spinach, pork, and lima beans. He was treated with the inhalants to which he was found sensitive and the offending foods were withdrawn from his diet, with fairly good control of his paroxysmal bouts of asthma. However, in December, 1948, he became markedly dyspneic, particularly at night, and the cough increased. Re-evaluation of his status on December 23, 1948, indicated that from an allergic point of view, there was no change and so the possibility of an early left ventricular failure was entertained. He was given 2 cc. of mercuhydrin subcutaneously with clearing of his nocturnal dyspnea and a loss of 4 pounds in weight. Thereafter, he was placed on a maintenance dose of mercuhydrin, depending on his dry weight, with control of the pulmonary signs and symptoms.

Case 5. C. A., a 64-year-old white male, was admitted to the Fourth Division Allergy Clinic of Bellevue Hospital in 1945, complaining of severe bronchial asthma and hay fever of 9 years' duration. His past history disclosed angina on effort for many years, but no episodes of congestive failure. There was a family history of allergy.

Physical examination revealed moderate dyspnea but no orthopnea and slight cyanosis of the lips. The chest appeared emphysematous with increase in the anterior posterior diameter. The heart was enlarged to percussion and the lungs were hyper-resonant. There was a faint systolic murmur at the base and the rhythm was regular. The blood pressure was 130/74. There were many rhonchi, wheezes and sonorous rales scattered throughout both lung fields. The liver and spleen were not enlarged and there was no peripheral edema.

Roentgen examination of the chest revealed enlargement of the cardiac silhouette in the transverse diameter, with dilatation of the ascending aorta, and the lungs showed fibrosis and emphysema. Roentgenograms of the nasal sinuses showed thickening of the mucous membranes. The electrocardiogram was typical of left ventricular strain.

The red blood count was 5.7 million cells per cc., and the hemoglobin was 15 gm. There were 8400 white blood cells and the differential was normal. The blood chemical studies, serologic tests and urinalysis demonstrated no abnormalities. Intracutaneous tests were positive for dust, ragweed, orris root, kapok, pork, onion, tomato and celery.

With proper diet and hyposensitization therapy, the asthma and hay fever were under control until January 13, 1949. At this time, his pulmonary symptoms reappeared and failed to respond to repeated doses of adrenalin and aminophyllin. Re-examination disclosed marked dyspnea, orthopnea, and cyanosis. There were many rhonchi and rales in both lung fields. The liver was not palpable, nor was there any peripheral edema. Subcutaneous injection of 2 cc. of mercuhydrin was given, and on the following day a loss of 4½ lbs. in weight was noted in addition to considerable clinical improvement. Thereafter, he was placed on a maintenance mercuhydrin therapy with control of the asthmatic paroxysms.

Case 6. J. K., a 72-year-old white male, was admitted to the Fourth Division Allergy Clinic of Bellevue Hospital in 1942, complaining of hay fever of 30 years' duration and bronchial asthma for 15 years. There was a family history of allergy. There was a history of angina on exertion but no previous episode of congestive failure.

On physical examination, dyspnea and cyanosis were apparent. The chest was emphysematous with flaring of the ribs and fixation of the diaphragms. Cardiac dullness was somewhat enlarged to the left. The heart sounds were distant, the rhythm was regular and no murmurs were heard. The blood pressure was 160/90. Many sonorous, sibilant rales and wheezes were heard throughout both lung fields. The liver and spleen were not palpable. The extremities revealed no peripheral edema, but clubbing of the fingers and toes was present.

Roentgen examination of the chest disclosed enlargement of the heart to the left with fibrosis and emphysema of the lungs. The sinuses demonstrated thickening of the mu-

cous membranes on Roentgen-ray examination. The electrocardiogram showed evidence of left ventricular strain.

His blood count was 5.5 million red cells, with a hemoglobin of 15.5 gm. The white blood cells numbered 7400 and the differential was within normal limits. The urinalysis disclosed a specific gravity of 1.018 with no albumin or sugar and on microscopic examination no cellular elements were seen. The blood chemical and serological tests were negative.

Cutaneous tests were positive to timothy, ragweed, dust, orris root, rabbit hair, chicken, green peas and bananas. Numerous antral punctures revealed no pus, nor were there any organisms grown on culture from the slight exudate obtained. On proper hypoimmunization therapy and avoidance of the symptom producing foods, there was considerable improvement. However, in January, 1949, this patient complained of severe asthma, particularly at night, and failure to respond to the usual drugs that formerly gave him relief. On re-examination there was no evidence of any additional hypersensitive factors, so 2 cc. of mercuhydrin was given subcutaneously. The next day there was a loss of 3½ lbs. in weight, with considerable improvement of symptoms. Thereafter, he was placed on maintenance mercurial therapy with amelioration of the asthmatic episodes.

Comment. All 6 cases presented were males whose ages varied from 59 to 71. There was a definite family history of hypersensitivity in 5 and an associated allergic disease, hay fever, in 3. Positive intracutaneous tests to numerous inhalants and foods were elicited in every case. Pulmonary fibrosis and emphysema were present in all, and the duration of the bronchial asthma was from 5 to 15 years. Hypertension was apparent in 4 of the cases and arteriosclerotic heart disease was present in the entire group. The dyspnea, cough, wheezing and rales in the chest that reappeared in these patients while under therapy for their long standing bronchial asthma, might easily have been interpreted as a recurrent paroxysm of the allergic asthma, especially since there was no past history of frank congestive failure. However, inasmuch as there was some evidence of cardiac disease, the possibility of early left ventricular failure was entertained, and this led to the use of a mercurial diuretic.

Mercuhydrin was injected subcutaneously in the gluteal region with a 26-gauge needle without any discomfort or pain. The patients were weighed before and after therapy in order to determine the dry weight and maintenance dose[8].

The control group consisted of young adults between the ages of 25 to 40 without any evidence of cardiac involvement. They fulfilled all the criteria necessary for the diagnosis of allergic bronchial asthma. They were weighed before and after mercuhydrin injections, and although a loss of 1 to 2½ lbs. was noted in 24 hours, the asthmatic paroxysm was unaffected.

Left ventricular failure is frequently ushered in by a paroxysmal acute dyspnea generally occurring at night but sometimes during the day, either at rest or after exertion, lasting from minutes to hours and accompanied by cough, wheezing and a sense of suffocation. On physical examination it closely resembles allergic bronchial asthma which is considered to be a form of paroxysmal dyspnea with signs of wheezing, sibilant and sonorous rales, and rhonchi[3]. In the uncomplicated case the history, the age of the patient, the Roentgen evidence of cardiac enlargement, the electrocardiographic manifestations of myocardial damage, the demonstration of positive skin tests and the presence of eosinophils in the bronchial secretions help to identify the correct etiology. However, when both conditions exist simultaneously, as in the above cases, these criteria are not very helpful.

Considerable importance has been attached to the circulation time by Ungar[13] and Plotz[11] in an attempt to differentiate cardiac failure from bronchial asthma. Many observers[1,2,4,7,9,12] found it definitely elevated in

left ventricular failure but normal in bronchial asthma *per se*. There is no doubt that this test is a valuable aid in diagnosis, especially when both conditions co-exist, yet occasionally, there may be a normal circulation time in early left ventricular failure and often it cannot be performed at the time most needed.

The therapeutic response to various drugs has been frequently employed in the differential diagnosis of certain diseases. The dramatic relief obtained in congestive heart failure by morphine is well known. In allergic bronchial asthma, however, it may be extremely dangerous, negating its potential diagnostic value. It has been frequently stressed that the favorable response of bronchospasm to adrenalin connoted an allergic etiology. Inasmuch as Plotz has shown that bronchospasm also exists in pulmonary congestion resulting from left ventricular failure and that it may also clear following the use of adrenalin, this concept becomes invalid. Aminophyllin, a powerful relaxer of smooth muscle when given intravenously, controls both types of asthmatic paroxysms; hence, its use in the mixed type of case sheds little light. Digitalis is an excellent therapeutic agent in congestive heart failure so that a favorable response to it might indicate a cardiac basis. Yet, there are some cases of high output failure that are uninfluenced by this cardiotonic drug, so that it cannot be used effectively in all cases. Mercuhydrin on the other hand, is a drug that is extremely effective in all types of congestive failure. Gold and his co-workers[6] have stressed its importance and demonstrated its usefulness not only in cases of frank congestive failure but also in early left ventricular failure with minimal signs. Hence, a positive therapeutic response to its use may be of diagnostic value.

The clearing of the asthma in the above described patients, following the subcutaneous injection of mercuhydrin at a time when the previously successful anti-allergic regimen failed, suggested a cardiac etiology. This was supported by the fact that evidence of cardiac disease existed in all 6 cases and by the fact that the asthmatic paroxysm occurring in the control group without heart disease failed to respond to mercuhydrin. The cardiac failure with its resultant fluid retention and pulmonary edema may either have aggravated the allergic edema and rendered it refractory to the anti-allergic therapy, or it may well represent a distinct bout of left ventricular failure not related to the pre-existing asthma.

Nevertheless, it appears clear that in allergic patients over 50 years of age in whom pulmonary signs and symptoms of bronchospasm fail to respond to proper hyposensitization therapy, the possibility of a cardiac factor must be considered and treated. In such instances, mercuhydrin succeeds in clearing the pulmonary manifestations of heart failure which, on physical examination, cannot be distinguished from bronchial asthma.

Summary. 1. Six patients over 50 years of age with long standing allergic bronchial asthma and positive skin tests, who suddenly became refractory to anti-allergic therapy, are presented.

2. All of the patients had evidence of cardiac disease but no history of frank congestive failure.

3. Subcutaneous injection of 2 cc. of mercuhydrin at the time of the recurrence of the asthmatic paroxysm caused clearing of the pulmonary signs and symptoms.

4. A control group of 20 patients with uncomplicated allergic bronchial asthma whose ages varied from 25 to 40 years were treated with subcutaneous mercuhydrin without success.

5. The positive therapeutic response of the above cases to mercuhydrin indicated a cardiac etiology.

6. Mercuhydrin was useful in the recognition of a cardiac factor complicating bronchial asthma.

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TERRAMYCIN: CLINICAL, PHARMACOLOGIC, AND BACTERIOLOGIC STUDIES

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Terramycin, a yellow crystalline antibiotic isolated from *Streptomyces rimosus*, has been shown to exhibit a wide range of antimicrobial activity[2]. This agent is available clinically in the form of the hydrochloride.* It may be administered orally or intravenously, and is relatively stable over a wide range of temperature and pH.

The purpose of this report is to present bacteriologic and pharmacologic investigations of terramycin, and to summarize our experiences in the use of this agent in the management of infections commonly encountered on the medical and pediatric wards of a general hospital.

Bacteriologic Studies. *Bacterial Sensitivity.* Tests for bacterial sensitivity were performed by means of a tube serial dilution method, using a 10^{-4} dilution of an 18 hour culture. The results are shown in Table 1.

The majority of the organisms tested were highly sensitive to terramycin. An important exception occurred with *E. coli*, in which 16 of 47 strains tested grew in concentration of 50 μgm. per cc. or higher. No strains of *B. proteus* were tested, and the only strain of *Ps. aeruginosa*, which occurred in a mixed infection with *E. coli*, was inhibited by 6.25 μgm. per cc. Bacterial resistance developed during therapy in 4 patients with urinary tract infections due to *E. coli*.

Pharmacologic Studies. Serum terramycin assays were performed in this laboratory by means of a serial dilution method, utilizing *B. cereus* as the test organism.

Terramycin administered orally was absorbed rapidly with the appearance of antibacterial activity in the serum within 1 hour of administration. No significant difference was noted in the serum concentrations when this agent was given to patients in the fasting or the non-fasting state. Serum concentrations observed in 10 individuals after the administration of a single oral dose of 1 gm. are presented in Fig. 1.

Most individuals attained a maximal concentration of 2.5 to 10 μgm. per cc. after 2 to 3 hours; this level was maintained for about 4 hours and

* We are indebted to Dr. Gladys L. Hobby of Chas. Pfizer & Co., Inc. for the terramycin used in this study.

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thereafter the concentrations steadily declined so that 0.1 to 0.5 μgm. per cc. was present at the end of 24 hours. Despite the presence of detectable amounts of terramycin in the serum for 24 hours, no cumulative effect was noted after repeated daily doses of 1 gm. or after the administration of 1 gm. every 6 hours. Individuals who received 1 gm. of terramycin every 6 hours usually maintained blood levels of 5 to 10 μgm. per cc. throughout the 24 hour period.

A satisfactory intravenous preparation of terramycin has recently become available. The serum concentrations of 10 individuals who received 250 mg. intravenously are presented in Fig. 2.

Most individuals had a serum level of 5 to 10 μgm. per cc. at the end of 1 hour, and at 12 hours 1 to 5 μgm. per cc. was present.

CLINICAL MATERIAL. A total of 108 cases was treated with terramycin. Twenty-eight of these had urinary tract infections, 38 had bacterial pneumonias, 21 had pertussis, 6 had tonsillitis, 4 bacillary dysentery, and 11 had miscellaneous infections including 1 case of amebiasis, 1 of typhoid fever, and one of subacute bacterial endocarditis.

TABLE 1.—*IN VITRO* SENSITIVITY OF VARIOUS BACTERIA TO TERRAMYCIN

Organism	*Inhibited by 0.25 μ gm./cc. or less* No. of cultures	*Inhibited by 0.25–1.6 μ gm./cc.* No. of cultures	*Inhibited by 1.6–12.5 μ gm./cc.* No. of cultures	*Inhibited by 12.5–50 μ gm./cc.* No. of cultures	*Inhibited by Greater Than 50 μ gm./cc.* No. of cultures
E. coli	0	2	25	4	16
Paracolon bacillus	0	0	1	0	0
Ps. aeruginosa	0	0	1	0	0
Shigella Flexner	0	3	1	0	0
S. typhosa	0	0	2	0	0
H. influenzæ	2	2	1	0	0
Friedländer bacillus	0	1	0	0	1
Strep. viridans	0	1	0	0	0
C. diphtheriæ	1	0	0	0	0
Pneumococcus	91	3	0	0	0

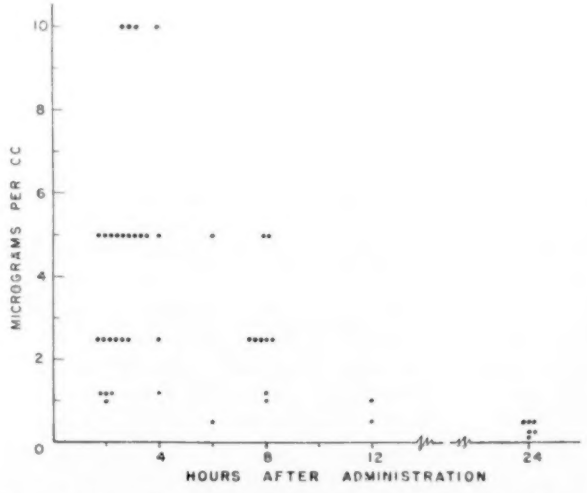

FIG. 1.—Serum terramycin concentrations after oral administration of 1.0 gm.

URINARY TRACT INFECTIONS. Twenty-eight cases of urinary tract infection were treated with terramycin. These may be divided into several clinical groups for purposes of evaluating the effectiveness of the drug. In the entire group of 28, a good clinical response was observed in 24, and there was at least temporary sterilization of the urine in 20 patients. No patients were followed for more than 1 week after stopping terramycin, so that information concerning the rate of relapse is not available.

Acute Infections. Eight patients had upper urinary tract infections with an acute onset of dysuria, chills, fever, and costo-vertebral angle tenderness. In no case was a bacteremia demonstrated. All 8 had an excellent clinical response, although in 2 cases urine cultures remained positive. In one, a 9-year-old girl with acute pyelonephritis caused by a terramycin sensitive strain of *E. coli*, a minimal bacilluria persisted. In the other, a 20-year-old male paraplegic with an indwelling catheter, the organisms were resistant to terramycin prior to therapy; acute symptoms promptly subsided, but the urine remained infected. In the remaining 6 patients, all of whom had sensitive organisms, urine cultures obtained 48 hours after therapy were sterile.

Three patients with acute lower urinary tract infections due to sensitive organisms were treated, with an excellent clinical response, but with persistence of a few colonies of *E. coli* in one patient in whom a marked depression in terramycin serum levels resulted from simultaneous amphojel administration.

Chronic Infections. Seventeen patients were treated, of whom 5 had chronic pyelonephritis and 12 had chronic infections apparently localized to the lower urinary tract.

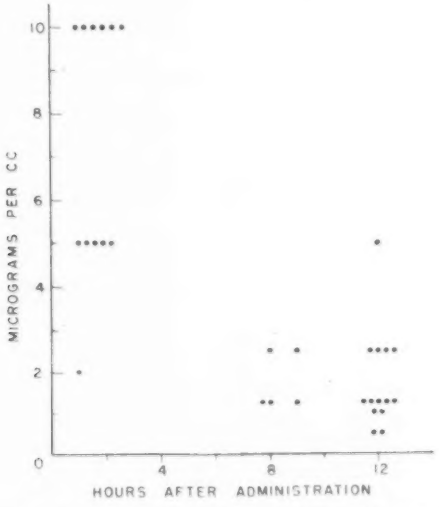

FIG. 2.—Serum terramycin concentrations after administration of 250 mg. intravenously.

Of the 5 patients with definite upper urinary tract involvement, the urine of 3 became sterile during treatment. Of these 3 patients, 2 were infected with sensitive strains of *E. coli*, and one with an *E. coli* - *Ps. aeruginosa* mixed infection in which both organisms were terramycin sensitive. One patient with terminal uremia and chronic *E. coli* pyelonephritis failed to improve on therapy, and one man with an enlarged prostate and urinary re-

tention associated with a chronic *E. coli* infection did not respond. The patient with terminal uremia had a resistant organism which grew in more than 200 μgm. of terramycin per cc. before therapy, while the man with prostatic enlargment developed a resistant strain during treatment.

Of the 12 patients with chronic infections localized to the lower urinary tract, 9 had sterile urine cultures 48 hours after therapy was stopped, while in 3 patients with chronic *E. coli* infections, one of whom also had advanced cirrhosis, there was no improvement. Of the 9 who responded satisfactorily, 7 were initially infected with sensitive organisms, while 2 patients were infected with strains of *E. coli* which were resistant *in vitro* to 50 μgm. per cc. of terramycin before treatment. Of the 3 who did not respond well, 1 was initially infected with a resistant organism, while another developed a resistant organism during therapy.

Method of Treatment. The daily dosage of terramycin in adults was in all cases 4 gm., given as 1 gm. every 6 hours. The duration of treatment was 4 to 6 days in 24 of the 27 adult cases, while 2 patients received the antibiotic for 3 days and one for 8 days. The one child, a 9-year-old white girl with acute pyelonephritis, received 250 mg. every 4 hours for 5½ days.

Bacteriologic Features of Urinary Tract Infections. Twenty-four single infections and 4 mixed infections were treated. Of the single infections, 23 were due to *E. coli* and one was due to the paracolon bacillus; of the 4 mixed infections, 2 were due to *E. coli* plus *A. aerogenes*, one to *H. strep* and *Staph. albus*, and one to *E. coli* and *Ps. aeruginosa*.

Of the 23 single *E. coli* infections, 15 cleared completely with disappearance of pyuria and sterilization of urine, 2 improved satisfactorily although a minimal bacilluria persisted, 2 improved clinically but cultures remained positive, and 4 were unchanged after therapy. One case of acute pyelonephritis due to the paracolon bacillus responded with urinary sterilization. The urine of all 4 patients with the mixed infections became sterile, including that of the patient with the *E. coli - Ps. aeruginosa* infection.

PERTUSSIS. Twenty-one cases of pertussis were treated in children varying in age from 3 months to 7 years. Five of these had an associated bronchopneumonia. The diagnosis of pertussis was made on the basis of epidemiological and clinical evidence, and lymphocytosis on the blood smear, since bacteriological facilities were inadequate for the isolation of *H. pertussis*.

Of the 5 patients with an associated bronchopneumonia, an excellent clinical response was observed in 2, while 2 patients responded only moderately well, and one severely ill 2-year-old white male with an associated pertussis encephalitis was placed upon intravenous aureomycin, when after 48 hours of oral terramycin therapy his condition continued to deteriorate. His illness remained unchanged for 2 days, and thereafter slowly improved on aureomycin therapy.

Of the 16 children with uncomplicated pertussis, an excellent clinical response was observed in 12, while a slow response was noted in 2, and 2 infants with severe paroxysmal coughing did not improve after 6 days of treatment and were then given aureomycin with subsequent gradual clinical improvement. In the patients who received both terramycin and aureomycin, there was no definite evidence that the response to aureomycin was better than to terramycin.

Method of Treatment. The daily dose of terramycin varied, depending upon the age and weight of the child, from a total of 200 mg. per day given in 4 divided doses in the youngest, to 1.0 gm. per day given as 250 mg. every 6 hours in the oldest patient. The ma-

jority of the patients received 125 mg. every 4 or 6 hours, and were treated for 4 to 7 days, although one child was given the antibiotic for only 2 days and one received it for 14 days.

Pneumonia. Twenty-five bacterial pneumonias in children were treated with oral terramycin. Thirteen adult patients with bacterial pneumonia were treated either with the intravenous terramycin preparation alone, or were begun upon intravenous therapy and then continued on oral medication for the duration of their therapeutic course. The intravenous preparation was employed in an attempt to evaluate its clinical effectiveness, not because of the severity of the disease.

Of the 25 pneumonias in children, a pneumococcus was isolated from 3, and of the 13 adult pneumonia cases, pneumococci were recovered and typed in 9. There were no bacteremias in children, and there was 1 positive blood culture (Type 12) in an adult.

Of the 25 pediatric cases, 20 made uneventful recoveries with striking clinical improvement within 48 hours, and often within 24 hours. A less striking response was observed in 2 infants with a persistent bronchiolitis. One 6-month-old infant with a severe bronchiolitis did not improve and was placed upon penicillin with subsequent improvement. A 7-year-old boy with a pneumococcal pneumonia who received 3½ days of therapy responded well, but was readmitted 3 days after discharge with a relapse of his right lower lobe pneumonia. A 5-year-old girl with pneumonia and pleural effusion showed no temperature response with terramycin, or subsequently with penicillin and sulfadiazine. A loculated empyema was eventually drained, and no organisms were cultured from the pus.

Of the 13 bacterial pneumonias in adults, 10 responded with prompt clinical and Roentgen-ray improvement. Three patients, one of whom had a Type 12 pneumococcal bacteremia, developed sterile pleural effusions while receiving therapy, and ran a more protracted but essentially uncomplicated course.

Method of Treatment. The therapeutic regimen for children was the same as that previously outlined for pertussis. Patients received the antibiotic in a powder, or as a cherry-flavored elixir, usually for 5 to 7 days.

Intravenous terramycin was given to the adults in 250 mg. amounts, dissolved in 100 cc. of 5% glucose, every 12 hours. Adults on oral therapy received 4 gm. per day in 1 gm. doses. The majority of adult patients received either parenteral, or combined parenteral and oral, therapy for 5 days.

Tonsillitis. Six cases of acute tonsillitis in children were treated for an average of 4½ days. An excellent response was noted in all six within 48 to 72 hours, with rapid subsidence of temperature and physical findings.

Bacillary Dysentery. Two cases of acute bacillary dysentery in children due to Flexner Type 2 and Type 3 organisms, respectively, were treated for 9 days and showed a marked clinical improvement within 72 hours, with disappearance of toxic symptoms and bloody diarrhea. Two other individuals, one an adult and one a child, who were carriers of Shigella Type 2 organisms, were also treated. After 5 days of therapy, repeated stool cultures were negative.

Miscellaneous Infections. A total of 11 miscellaneous cases was treated. Adults received 1 gm. of terramycin every 6 hours, while children received doses of similar magnitude according to weight.

Amebiasis. A 52-year-old white male with intestinal and hepatic amebiasis was treated with 4 gm. of terramycin daily for 9 days. At the end of this time his stools were more formed and his liver less tender, although cysts and trophozoites of *E. histolytica* were still

present and his temperature elevation and hepatic enlargement persisted. Terramycin was therefore discontinued and he was begun upon other antiamebic therapy. Shortly thereafter, he died of disseminated coccidioidomycosis. It seems probable that this accompanying disease was an important factor in the relatively poor response of the amebiasis to terramycin.

Subacute Bacterial Endocarditis. A 51-year-old colored male with subacute bacterial endocarditis due to *Strep. viridans* was treated with 4 gm. of terramycin per day for 14 days. Daily blood cultures were positive for 5 days after therapy was begun, and thereafter were negative. The temperature was normal after 5 days of treatment, but he remained listless, anorexic, and nauseated while receiving terramycin. Terramycin was therefore discontinued and he has made a favorable response to penicillin therapy.

Typhoid Fever. A 52-year-old white male plumber, moderately ill for one week, was found to have blood and stool cultures positive for *S. typhosa.* He was treated with a total of 65 gm. of terramycin in 16 days. After 3 days his temperature became normal, and blood and stool cultures taken on the 7th day were negative. His disease relapsed 10 days after therapy was completed, and he was re-treated with 4 gm. of terramycin per day for 17 days. He again made an excellent response, and has remained well. Stool cultures have remained negative during a follow-up period of 2 months.

Mumps. Two patients with mumps, both with an associated orchitis, were given 4 gm. of terramycin per day for 4 and 5 days respectively. Parotid and testicular tenderness and swelling subsided promptly during therapy, but evaluation of these results is difficult in view of the variable course of the disease.

Infectious Mononucleosis. A 23-year-old male with infectious mononucleosis was ill with a high temperature for 2 weeks despite therapy with penicillin and chloromycetin. His temperature returned to normal within 24 hours of starting terramycin. Here again this may have been a coincidence.

Gonorrhea. One patient with acute gonococcal urethritis showed marked symptomatic improvement within 12 hours after receiving 1 gm. of terramycin. He was given another gram the next day, and cultures thereafter were negative.

Diphtheria. One patient with diphtheria requiring a tracheotomy received terramycin for 10 days as well as antitoxin. Cultures following therapy were negative.

Bronchiectasis. A 65-year-old man with chronic bronchiectasis received 4 gm. of terramycin daily for 6 days, and noted dramatic subjective relief with a marked diminution in the amount of his sputum after 3 days.

Lung Abscess. In a 45-year-old man with a large lung abscess, there was a marked decrease in sputum and symptoms following the administration of 16 gm. of terramycin in 4 days. He later received other antibiotics, and the abscess cavity closed within 3 weeks.

Erysipelas. A man with erysipelas exhibited a striking improvement within 24 hours after therapy was begun, and recovery was complete within 5 days.

Discussion. It is apparent from these studies that terramycin possesses a wide range of antibacterial action against many of the more common gram-positive and gram-negative pathogens. The antibiotic is usually administered by mouth, although an intravenous preparation has recently become available. As with aureomycin, absorption from the intestinal tract is incomplete; blood levels observed following oral administration are similar in magnitude to those attained by the injection of only one fifth to one tenth

as much intravenously. Toxic reactions to the drug taken orally are uncommon and rarely severe; they consist of nausea, vomiting, and diarrhea. Toxic manifestations did not occur in the 10 patients in our series in whom the intravenous compound was employed.

With both oral and intravenous routes, terramycin blood levels are similar in magnitude to those obtained with aureomycin, and are considerably lower than those observed with chloramphenicol. This is of interest in view of the fact that aureomycin deteriorates rapidly, whereas terramycin is relatively stable. It would appear that terramycin and aureomycin are excreted at approximately the same rate, and that chloramphenicol is eliminated from the body somewhat more slowly. In patients who received 1 gm. of terramycin orally, significant amounts remained in the serum after 24 hours. There was no cumulative effect with the prolonged administration of 1 gm. each day, or at intervals of 6 hours.

Results obtained in the treatment of urinary tract infections with terramycin illustrate many features previously observed with aureomycin and chloramphenicol. Almost all of the patients manifested decreased pyuria and bacilluria during the period of chemotherapy, whether or not urinary sterilization was obtained. In the presence of anatomic obstruction, foreign bodies, or chronic infection, relapse following initial improvement was common. This is not surprising in view of the fact that terramycin is primarily bacteriostatic in its action. It is of interest that in the present series terramycin resistant strains of *E. coli*, which grew in 50 μgm. per cc. or more, were recovered from 5 of 28 patients prior to therapy. In addition, in 4 patients resistance developed during the course of therapy. Development of bacterial resistance has also been recently described in the treatment of some patients with urinary tract infections with aureomycin and chloramphenicol[1]. Most strains of *B. proteus* and *Ps. aeruginosa* have been found to be resistant *in vitro* to terramycin, as well as to aureomycin and chloramphenicol. The one strain of *Ps. aeruginosa* encountered in the present study was quite sensitive *in vitro*, and the patient responded well to therapy.

The response in 18 of the 21 cases of pertussis indicates that terramycin is effective in diminishing the severity of this disease, and in controlling the pulmonary complications which are occasionally severe and may be fatal. These results with terramycin are in all respects comparable to previous therapeutic experience with aureomycin or chloramphenicol.

The results obtained in bacterial pneumonias in children and in adults indicate that terramycin is an effective therapeutic agent in this disease, and produces clinical improvement comparable to that seen with penicillin, aureomycin, or chloramphenicol. The results in a patient with bronchiectasis suggest that terramycin is of benefit in controlling acute exacerbations, and may be of value in diminishing chronic pulmonary symptoms.

In the group of miscellaneous diseases treated, it would appear that the antibiotic is an effective agent in the chemotherapy of acute tonsillitis, acute lung abscess, acute and chronic bacillary dysentery, acute gonococcal urethritis, and erysipelas. The patient with typhoid fever appeared to respond as well to terramycin as the majority of cases do to chloramphenicol. However, conclusions concerning the relative effectiveness of the two antibiotics can obviously not be made on the basis of one case. In the patient with subacute bacterial endocarditis, blood cultures remained positive for 5 days after terramycin was begun, although the patient improved clinically. It is probable that the bacteriostatic action of terramycin will render this antibiotic less effective in the eradication of this infection than penicillin,

which causes actual killing of bacteria. Contrary to experience elsewhere[3], results in our patients with amebiasis were relatively poor. Clinical improvement in 2 patients with mumps and 1 with infectious mononucleosis indicates the need for further evaluation of this antibiotic in the therapy of virus infections in general.

Summary and Conclusions. 1. One hundred and eight cases of infections commonly encountered on the adult and pediatric medical wards of a general hospital were treated with terramycin. Adults received 1 gm. of terramycin orally every 6 hours, and children were given doses of similar magnitude according to weight. Ten adults with pneumonia received 250 mg. of an intravenous preparation twice a day. Toxic reactions, consisting of nausea, vomiting, and loose stools, were uncommon, and in only one instance were they severe enough to require discontinuing the drug.

2. Blood levels of 2.5 to 10 μgm. per cc. were maintained for 6 to 8 hours in adults after the oral administration of 1 gm.; thereafter they declined, with levels of 0.1 to 0.5 μgm. per cc. remaining in the majority of patients after 24 hours. Patients who received 1 gm. every 6 hours maintained levels of 5 to 10 μgm. per cc. throughout the day. Levels of 5 to 10 μgm. per cc. were present 1 hour after the administration of 250 mg. of the intravenous compound, and after 12 hours, 1 to 5 μgm per cc. persisted.

3. Clinical improvement was noted in 24 of 28 patients (85%) with urinary tract infections, and there was at least temporary urinary sterilization in 20 (71%). Resistant strains of *E. coli* were isolated from 5 patients before therapy. In 2 of these patients, urine cultures were sterile after treatment; in 1 there was marked clinical improvement but bacilluria persisted, and in 2 there was no improvement. In addition, 4 patients developed drug resistance during treatment. No cases of infection with *B. proteus* were treated, and 1 patient with a *Ps. aeruginosa* had a sensitive organism *in vitro* and responded well to therapy. These results are comparable to those obtained with other antibiotics and are influenced largely by the same factors as determine cure with these other agents.

4. Eighteen of 21 children (86%) with pertussis responded to terramycin with clinical improvement. Five of these had an associated bronchopneumonia, which cleared in 4. These results indicate that terramycin is as effective an agent in the therapy of pertussis as chloramphenicol or aureomycin.

5. Favorable results were observed in 22 of 25 pneumonias in children, and in all of 13 adults with bacterial pneumonias.

6. Favorable responses were noted in patients with bacillary dysentery, tonsillitis, erysipelas, and bronchiectasis. A patient with typhoid fever had an excellent response to terramycin, both during the initial attack and during relapse. In 1 patient with subacute bacterial endocarditis, and another with amebiasis, definite clinical improvement was noted, although in both instances it was necessary to change to other chemotherapeutic agents. Two patients with mumps complicated by orchitis, and one with infectious mononucleosis, improved promptly when terramycin was administered.

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CONFUSING ASPECTS OF INFECTIOUS MONONUCLEOSIS

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THE disease entity infectious mononucleosis has been defined by the triad of a peculiar hyperplasia of the lymphoid system[9], the presence of circulating abnormal lymphocytes[12], and the appearance in the blood serum of agglutinins for sheep cells which are absorbed by ox cells but not by guinea pig kidney[10,30]. The subject has been extensively reviewed[5,34]. However, the clear cut entity is surrounded by an aura of findings which raise questions that must remain unanswered until the etiologic agent is discovered and more is known regarding the host response to this agent. The manifestations of the disease are protean, and, although such complications as electrocardiographic changes and central nervous system or pulmonary involvement[34] are generally recognized, the asymptomatic form of the disease has been little stressed, and it is in this realm that diagnostic confusion arises. This paper describes what may be considered a "subclinical" epidemic and reviews other such epidemics. In addition, certain diagnostic and prognostic difficulties associated with the finding of the abnormal mononuclear cells are discussed.

Materials and Methods. A program was set up to study possible hematological changes associated with cold exposure, in men aged 19 to 35 years transported to a field laboratory at Churchill, Manitoba, Canada, from the Quartermaster Climatic Research Laboratory at Lawrence, Massachusetts, during the winter of 1947-48. The discovery of abnormal mononuclear cells in many of the blood films, consistent with those seen in infectious mononucleosis, prompted a close observation of the entire group for any symptoms of the disease. Blood films were taken on 12 men before their departures from Lawrence at various times between October and December, and at intervals of at least once a month during the winter until they all returned to Lawrence at the end of March. When the widespread appearance of abnormal cells was noted in this group, blood films were taken on the 14 other members of the unit, on February 16-18. These were repeated on all members of the unit remaining at Churchill on March 1 and 22, and on those men available on return to Lawrence on either April 22 or June 9. Simultaneously total leukocyte counts and heterophile agglutinations were measured.

The blood films on cover slips were stained with Wright's stain in the conventional manner. Differential counts on the subclinical cases were made by Dr. R. W. Heinle (Lakeside Hospital, Cleveland, Ohio). Heterophile agglutination tests were performed by Dr. A. E. Feller (Lakeside Hospital) using the technique employed by the American Public Health Association[11].

Results. At some time during the winter every member of the group showed abnormal mononuclear cells, and the series is divided into 5 clinical and 21 subclinical cases. The clinical cases included 1 with hepatomegaly, jaundice, splenomegaly, and positive heterophile agglutinins, and 4 with negative agglutinins and minimal symptoms. These cases are summarized in Tables 1 and 2 and Figure 1. Splenomegaly, hepatomegaly, jaundice, lym-

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TABLE 1.—DATA IN 5 CASES OF INFECTIOUS MONONUCLEOSIS WITH CLINICAL SYMPTOMS

Case	Date abnormal lympho-cytes first noted	Days before onset of symptoms	Date of first symptoms	Duration of symptoms (days)	Symptoms Noted: Lymph-adeno-pathy	Fatigue and Anorexia	Malaise	Fever	Sore Throat	Icterus	Heterophile Agglutination
A	28 Oct.	30	28 Nov.	140 (including a relapse)	+ + +	+ + +	+ + +	+ + +	+	+ + (ceph. Floc. + + + +)	1:1280
B	19 Nov.	31	20 Dec.	21	+	+ +	0	0	0	0	Neg.
C	19 Nov.	48	6 Jan.	42 (including a relapse)	+ +	+ + +	+	+	+ +	0	Neg.
D	17 Nov.	91	16 Feb.	60	+	+ +	+ +	0	0	0	Neg.
E	17 Nov.	131	26 Mar.	21	+ +	+ + +	+ +	+	0	0	Neg.

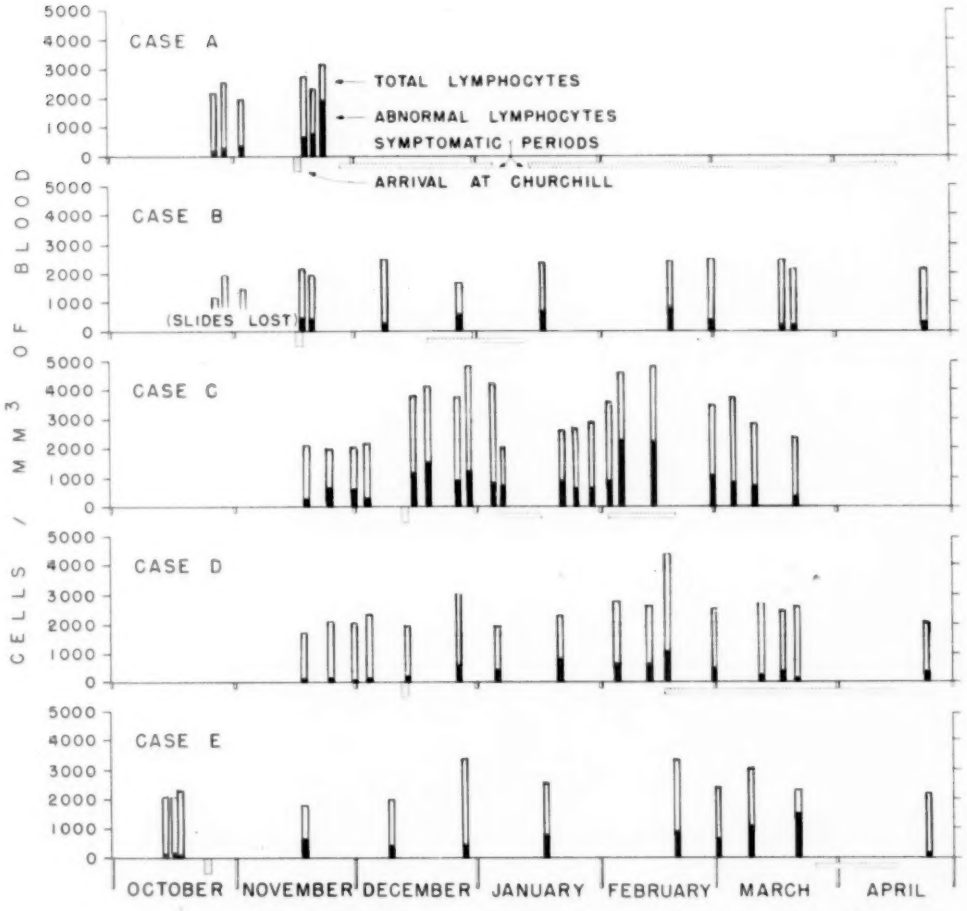

FIG. 1.—Total lymphocyte counts in symptomatic cases of infectious mononucleosis.

phadenopathy, rashes, and pharyngeal inflammation were sought for in all cases, but found only as noted above and in Table 1. All men were asymptomatic for 6 months before arrival at Churchill, and all denied a past history of infectious mononucleosis by name and symptoms. On blood films, the majority of the cells fell into the Downey[12] classification of type I and type II, with some variation in the characteristics that made it impossible to classify all of them definitely in one or the other group. A small percentage of cells containing nucleolus-like structures and of larger diameter, can be classified as type III of Downey, and there were a few large, dark-staining cells of striking appearance, of a type seen in this disease, resembling plasma cells[23].

TABLE 2.—LEVELS OF ABNORMAL CELLS IN 21 ASYMPTOMATIC CASES OF INFECTIOUS MONONUCLEOSIS

	Total White Cell Count			*% Normal Lymphocytes*			*% Abnormal Cells*		
Cases	*1 Mar*	*22 Mar*	*22 Apr*	*1 Mar*	*22 Mar*	*22 Apr*	*1 Mar*	*22 Mar*	*22 Apr*
F	8,500	8,450		3 0	7 0		30 0	15 0	
G	5,500	6,850		15 5	13 5		24 5	18 5	
H	6,800	10,100	8,250*	22 0	4 0	11 0*	23 0	38 0	6 0*
I	7,500	7,550	—	20 0	37 5	—	21 0	8 5	—
J	12,500	8,900	10,100	10 5	1 5	6 5	20 5	14 5	10 5
K	8,500	9,400	6,500	28 5	17 0	26 0	20 5	18 5	17 0
L	12,400	10,100	9,200	17 0	7 0	10 0	20 0	31 0	24 5
M	9,200	9,350	7,950*	18 5	11 5	—	16 5	16 5	—
N	9,300	12,650	12,500	17 5	4 5	19 5	13 5	17 0	4 5
O	7,300	12,050	—	19 5	10 5	—	13 5	12 0	—
P	7,800	8,450	—	35 0	17 0	—	12 0	27 5	—
Q	18,500	5,700	8,200*	15 0	48 5	12 0*	10 0	18 0	11 5*
R	7,800	—	6,800	32 5	—	26 0	8 5	—	15 0
S	6,400	5,900	9,100*	33 0	11 5	22 5*	8 0	25 0	7 5*
T	10,400	7,450	9,500	23 0	11 5	19 5	8 0	20 5	4 0
U	12,300	12,700	—	25 5	7 5	17 0*	7 5	20 5	1 0*
V	10,600	—	—	21 5	12 5	—	6 5	8 0	—
W	8,200	8,550	10,200	12 0	9 0	5 5	6 0	13 5	11 0
X	7,700	6,350	8,600*	25 5	10 0	15 0*	5 5	16 0	9 0*
Y	11,700	7,200	—	11 5	13 5		4 5	16 0	
Z	10,500	6,250	9,400	24 5	11 5	18 5	1 5	18 0	8 0

* Taken on 9 June.

Discussion. In examining the criteria of diagnosis on which the disease entity infectious mononucleosis is based, it is at once obvious that the first, reticulo-endothelial hyperplasia as judged by history and physical examination, is non-specific. Definition of the type of hyperplasia by node biopsy is in most instances unnecessary and excessively drastic. Also, as abnormal mononuclear cells may appear in the absence of demonstrable hyperplasia, the first criterion is of no value in equivocal cases.

The second criterion for diagnosis is the presence of abnormal mononuclear cells. These cells have been described frequently in other diseases, the most clear cut of which is infectious hepatitis[2,20,22,31] where in the experimentally produced disease[17] the cells generally appear during the first week. Likewise, the appearance of these cells in measles and German measles[4] has been frequently noted. These findings suggest that the abnormal mononuclear cells are a non-specific response to varying stimuli. When associated with the other 2 criteria in the triad (reticulo-endothelial hyperplasia and a positive heterophile agglutination) the abnormal cells offer no diagnostic problem. However, when found alone their significance becomes less well defined.

The third in the triad of criteria is

the positive heterophile agglutination. The value of this test when positive is undisputed, but a negative result cannot be used to exclude infectious mononucleosis. In reviewing the literature on this serological technique, Kaufman[21] noted that "the percentage of cases with positive agglutination tests varied from 43 to 100%". These discrepancies are accounted for by differences in techniques of recording dilutions, by time factors, end points, and frequency of sampling. For example, Barrett[3] obtained a frequency distribution curve of agglutinin titers in normal individuals significantly different from that obtained by Stuart[30], using the same technique. Also, the diagnostic criteria of the investigator may lead him to exclude cases solely because they do not show positive heterophile agglutinins. In this connection, Himsworth[18] reported 1 case in which significant antibody titer did not develop until 10 weeks after the onset of the disease.

Hence, if the criteria for the diagnosis of infectious mononucleosis are reticuloendothelial hyperplasia, abnormal circulating cells, and a positive heterophile agglutination, many pathological pictures appear which cannot be classified. The frequency of negative heterophile agglutination has been pointed out. The occurrence of only abnormal cells is not infrequent and introduces another aspect.

"Subclinical infectious mononucleosis" is a diagnosis which has been made when, in the absence of clinical symptoms, abnormal mononuclear cells with or without a positive heterophile agglutination are noted. These instances are generally associated with clinical cases in the population. If one assumes that the agent causing the overt cases, as defined by the triad, also causes those with only abnormal cells, then the group of cases described above could be considered an epidemic of subclinical infectious mononucleosis. Table 3 presents a résumé of the subclinical epidemics reported in the literature. It is of interest to note that in the above described series there were no positive heterophile agglutinations. Stevenson[29], using the Davidson technique and considering titers over 1:56 as positive, found 6.8% of his cases positive. Meer[24] found 17.6% with titers greater than 1:56. Halcrow[16], on the other hand, found 87% with titers of 1:32 or greater, using the Paul-Bunnel technique. From the above facts we may conclude that an entity exists, which in our present ignorance we call subclinical infectious mononucleosis.

The relation of subclinical to clinical cases is confusing, and in this vein it is interesting to note the latent period between the appearance of abnormal cells and the onset of symptoms. In the above series, in case A, this interval was at least 30 days; and in the other milder cases the interval varied from 31 to 131 days. Halcrow[16] and Immerman[19] mention the delayed appearance of symptoms, the latter noting abnormal cells "weeks or more" before the onset. This is in contrast to the usual description of the disease picture, in which the rise of abnormal cells occurs on the 4th or 5th day of illness[5], and in severe cases it has been noted that the appearance of abnormal cells may be longer delayed[32].

The latent period between cells and symptoms suggests the possibility of an agent-host relationship which is disturbed by external factors to produce clinical symptoms similar to the relationship which has been described for herpes simplex[7]. However, no correlation between appearance of symptoms and degree of physical stress or exposure to cold could be made, nor was percentage or absolute number of abnormal cells an index of value in predicting which cases would progress from an asymptomatic to a symptomatic stage. In a large population, this

type of widespread subclinical infection might explain the sporadic clinical cases.

In addition to the appearance of cells long before any clinical symptoms, it is known that they may persist long after symptoms have subsided. Farley[14] has described abnormal mononuclear cells present in the peripheral blood for 10 years after the initial "infection". host relationships are not understood.

Summary. 1. A mild clinical and subclinical epidemic most probably of infectious mononucleosis in a detachment of men sent to the arctic is reported. All 26 men showed abnormal mononuclear cells in their blood, while only 5 developed symptoms sufficient to classify them as clinical cases, and only 1 had a positive heterophile

TABLE 3.—RESUMÉ OF SOME PREVIOUSLY REPORTED EPIDEMICS OF INFECTIOUS MONONUCLEOSIS IN WHICH THERE WERE APPARENTLY NUMEROUS ASYMPTOMATIC CASES

		Cases with Abnormal Mononuclear Cells					
		Clinical Cases			*Subclinical Cases*		
Author	*Total persons examined*	*Total number*	*Number*	*Number with positive heterophile agglutinins*	*Number*	*Number with positive heterophile agglutinins*	*Type of Population*
Halcrow[16]	296	290	125	104	165	98	Occupants of an EMS hospital, England
	72	41	2	2	39	27	Persons in neighboring towns who had contact with the hospital
	29	0	0	0	0	0	Control group from hospital 35 miles away, no contact
Meer, Lutterloh, & Pilot[24]	—	89	26	—	63	—	Epidemic at Camp McCoy, Wisconsin, duration 6 months
	522	217	0	0	217	13 (of 74 examined)	Group of asymptomatic men examined during 6th month
Stevenson & Brown[28]	—	123	34	—	89	6	The asymptomatic group consisted of 73 hospital patients with other conditions, and 16 staff members
Randolph & Gibson[25]	—	24	—	—	24	0 (of 22 examined)	Persons complaining only of fatigue. One had infectious mononucleosis 2 years previously, 8 had history of cervical adenitis
Wechsler[34]	—	—	556	—	"During the height of the epidemic practically every patient in the hospital showed varying percentages of abnormal lymphocytes in their blood smears."		Cases at Ft. Bliss, Texas
Baldridge, Rohner & Hansmann[1]	67	67	12	—	55	—	University students, random sample of junior class taken during an epidemic
Guthrie & Pessel[12]	500	doubtful	112	—	about 188	—	Student body at a boys' school

Thus, the non-specificity of the cells, the subclinical aspects, the occasional appearance of cells long before symptoms, and the persistence of cells long after symptoms, make evaluation of the reports in the literature of sporadic appearance of abnormal cells in various diseases impossible[1,5,6,8,13,25,26,27,33,35].

It is thus evident that one cannot be dogmatic in defining criteria for diagnosis of the disease process, "infectious mononucleosis", when the etiological agent is unknown and the agent-

agglutination together with liver damage. Abnormal mononuclear cells were noted 30 to 131 days before the onset of symptoms in the 5 clinical cases.

2. The 3 generally accepted criteria for the diagnosis of infectious mononucleosis (reticuloendothelial hyperplasia, abnormal mononuclear cells, and a positive heterophile agglutination) are not always encountered together in symptomatic and asymptomatic individuals. The non-specificity of the cells

is pointed out, as well as the variability of the heterophile agglutination test.

3. The presence of only abnormal cells is discussed in terms of the nonspecificity of the cells, subclinical epidemics (literature reviewed), and the appearance of the cells before and persistence after symptoms.

4. Only when more is known of the etiological agent and the agent-host relationships will infectious mononucleosis be a well defined entity.

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THE INCIDENCE OF SICKLING

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The first report of a study of erythrocyte sickling in Philadelphia appeared in 1935[2]. Using the test tube method, of 100 Negroes tested, 13 were positive (incidence, 13%). This percentage was higher than that found in most areas of this country. A study, therefore, was made of 1000 Negro clinic and hospital patients at the Graduate Hospital of the University of Pennsylvania in order to determine a more significant figure for the incidence of erythrocyte sickling in this area. The literature was reviewed and the incidence of sickling in other regions was compiled.

Method. The moist stasis test of Scriver and Waugh[31] was used. After 5 minutes with a rubber band about the patient's finger, it was cleansed and pricked. A small drop of blood was taken on a cover slip which was placed, drop-side down, on a flat slide. The blood was allowed to spread with-

TABLE 1.—ERYTHROCYTE SICKLING IN THE NEGRO POPULATION OF THE UNITED STATES*

Year	*Author*	*Location*	*No. tested*	*No. positive*	*%*
1923	Sydenstricker *et al.*[34]	Georgia	300	13	4.3
1926	Cooley & Lee[10]	Michigan	400	30	7.5
1927	Lawrence[20]	Tennessee	100	5	5.0
1927	Miyamato & Korb[27]	Missouri	300	19	6.3
1928	Smith[32]	Louisiana	100	5	6.0
1928	Josephs[18]	Maryland	250	16	6.4
1928	Wollstein & Kreidel[39]	New York	150	13	8.7
1929	Levy[21]	New York	213	12	5.6
1929	Dolgopol & Stitt[12]	New York	77	4	5.2
1930	Graham & McCarty[15]	Alabama	1500	122	8.1
1931	Sydenstricker[35]	Georgia	1800	99	5.5
1932	Brandau[6]	Texas	200	13	6.5
1933	Diggs *et al.*[11]	Tennessee	2539	211	8.3
1933	Ahmann[1]	Flordia	674	65	9.6
1935	Beck & Hertz[2]	Pennsylvania	100	13	13.0
1936	Killingsworth & Wallace[19]	Texas	1205	65	5.3
1936	Hansen-Pruss[16]	North Carolina	100	14	14.0
1937	Cardoza[7]	Illinois	1263	119	9.4
1937	Johnson & Townsend[17]	South Carolina	719	57	7.9
1941	Tomlinson[35]	West Virginia	275	18	6.5
1943	Ogden[28]	Louisiana	692	45	6.5
1948	Watson *et al.*[38]	New York	226	18	8.0
1949	Margolies	Pennsylvania	1000	72	7.2
			Total 14,183	Total 1048	Mean 7.25

* In Watson's study[38] of the paucity of sickling in newborn infants, sickling tests were done on 226 consecutive newborn infants and their mothers. We have included here the incidence in adults, but have omitted the studies on infants. The inclusion of both would make one group reflect the other.

* Portion of thesis submitted to the faculty of the Graduate School of Medicine, University of Pennsylvania, in partial fulfillment of the requirements for the degree of Master of Medical Science in Internal Medicine.

out pressure, after which the edges of the cover slip were rimmed with petrolatum. Two to 3 preparations were made for each patient; those with air bubbles were discarded. The sealed drop preparations remained at room temperature and were examined under high-dry magnification of the microscope at 24, 48, and 72 hours. Sickling was reported as positive when typical sickle cell distortions and long tapering filaments were observed.

Results. Sickling was demonstrated in 72 of the 1000 patients examined, showing an incidence of 7.2%. Only 2 of this group were found to have sickle cell anemia.

Discussion. All of the incidence studies of sickling in the Negro race and its crossbreeds were reviewed. Of the 14,183 Negroes tested in the United States, 1043 showed positive sickling tests—an incidence of 7.25% (Table 1). In Africa, of 8668 Negroes tested, 962 were positive—an incidence of 11.1% (Table 2). In Central and South America, of 9016 Negroes tested, 861 (9.55%) were positive (Table 3). Totalling all the studies done in the world (Table 4), of 31,867 Negroes examined, 2871 were positive—an overall incidence of 9.01%.

TABLE 2.*—ERYTHROCYTE SICKLING IN THE NEGRO POPULATION OF CENTRAL AND SOUTH AMERICA

Year	*Investigator*	*Location*	*No. tested*	*No. positive*	%
1934	da Castro[8]	Brazil	86	11	12.8
1939	Chediak *et al.*[9]	Cuba	57	10	5.7
1943	Mera[25,26]	Colombia	577	54	9.4
1943	de Mendonca[23,24]	Brazil	703	48	6.8
1944	McGavack & German[22]	Honduras	300	24	8.0
1945	Tomlinson[36]	Panama	3000	246	8.2
1945	da Silva[30]	Brazil	1130	130	10.0
1946	Calero[6]	Panama	370*	51**	13.8***
1947	Boturao & Boturao[4]	Brazil	294†	20††	6.8†††
1949	Van der Sar[37]	Trinidad	2499	267	10.7
			Total 9016	Total 861	Mean 9.55

* 264 negroes, 106 mulattos
** 38 negroes, 13 mulattos
*** 14.3% negroes, 12.6% mulattos
† 162 negroes, 132 mulattos
†† 14 negroes, 6 mulattos
††† 8.6% negroes, 4.5% mulattos

TABLE 3.—ERYTHROCYTE SICKLING IN THE NEGRO POPULATION OF AFRICA

Year	*Investigator*	*Location*	*No. tested*	*No. positive*	%
1944	Evans[13]	West Africa	561	112	19.0
1944	Reid[29]	West Africa	100	13	13.0
1945	Altmann[1]	South Africa	403	1	.025
1946	Findlay *et al.*[14]	West Africa	5500	682	12.4
1947	Beet[5]	Rhodesia	2104	154	7.3
			Total 8668	Total 962	Mean 11.1

TABLE 4.—ERYTHROCYTE SICKLING THROUGHOUT THE WORLD

Location	*No. tested*	*No. positive*	%
United States	14,183	1048	7.25
Africa	8,668	962	11.1
South and Central America	9,016	861	9.55
	Total 31,867	Total 2871	Mean 9.01

The lower incidence of the sickling phenomenon in Negroes in the United States as compared with the studies done in Central, South America and Africa, may be due to the less pure strain of Negro blood found in this country.

The incidence of 7.2% found in Philadelphia compares favorably with the overall incidence of 7.25% for the United States.

Summary. 1. Sickling tests done on 1000 Negro in-patients and out-patients at the Graduate Hospital of the University of Pennsylvania revealed an incidence of 7.2%.

2. A review of all the available reported studies on the incidence of erythrocyte sickling reveals an incidence of 7.25% in the United States, 9.35% in Central and South America, and 11.1% in Africa, resulting in a world incidence of 9.01%.

3. The less frequent occurrence of sickling in the United States as compared with Central and South America and Africa may be attributed to the less pure strain of Negro blood found in this country.

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EFFECT OF TEMPERATURE ON SURVIVAL OF CHICK EMBRYOS INFECTED INTRAVENOUSLY WITH CRYPTOCOCCUS NEOFORMANS (TORULA HISTOLYTICA)

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Human torulosis is almost always fatal, since there is no effective remedy. The disease is caused by a yeast-like fungus, *Cryptococcus neoformans*, which characteristically produces meningitis. The organism is inhibited *in vitro* by numerous substances which are either ineffective *in vivo* or are too toxic for chemotherapeutic use[2].

Since among common laboratory animals the rabbit alone is not susceptible to infection, Kuhn suggested that the higher body temperature of the rabbit (102 to 104°F.) was unfavorable for the multiplication of the organisms[3]. In support of this hypothesis, Kuhn reported that when cultured *in vitro* at 39.4°C. the organisms were slowly killed, but at 40.6°C. (105°F.) most cells were dead within 24 hours. Kligman and Weidman confirmed these observations and noted that at 40°C. (104°F.) no viable cells remained after 6 days incubation[2]. These results suggested the possibility of using artificially induced fever as a therapeutic measure, especially since fever is generally not a prominent symptom of human torulosis. In fact, fever is usually minimal or absent in human torulosis[1]. Subsequently, Kuhn reported that infected mice maintained in an incubator room at 35 to 36°C. survived longer than those kept at room temperature[4]. The average temperature of the incubated mice was 103°F., but varied from 101 to 105°F. All of 15 intravenously infected mice were dead in eleven days at room temperature compared with only 4 of 15 mice maintained at 35 to 36°C. In our hands, however, attempts to maintain mice at a uniformly elevated temperature have failed. Either the diurnal fluctuations were too great or unexpected deaths occurred from hyperpyrexia. Among the unsuccessful methods employed were: 1) Maintenance in an incubator at various temperatures; 2) suspension of mice in constant temperature water baths after placing them in plaster of paris casts; and 3) maintenance in cages and special boxes heated by infra-red bulbs. It seemed desirable, therefore, to employ another susceptible animal with which to test the hypothesis that an elevation of body temperature to a level of 103 to 104°F. will cause *in vivo* death of *Cryptococcus neoformans*.

Since it was previously reported that chick embryos incubated at 37°C. are killed by *Cryptococcus neoformans* when injected intravenously in appro-

priate doses[2], it occurred to us that the temperature of the embryo might be artificially elevated sufficiently to kill the organisms without killing the embryo. The great majority of uninoculated embryos were found to survive until hatching when incubated at 40°C. from the 9th day of age. By plunging thermometers directly into the contents of embryonated eggs incubated at this temperature, it was learned that from the 9th to the 14th days the temperature of the egg is approximately ½°C. lower than the incubator temperature. From this time on the temperature slowly rises until on the 20th day the temperature of the egg is about 1°C. higher than that of the atmosphere. Although an error is thus introduced by the limited heat regulating capacity of the embryo, the temperature of the embryo does not actually rise above that of the incubator until several days before hatching. The error is small, furthermore, by comparison with the diurnal fluctuation of temperature in many mammals. The chick embryo was, therefore, considered to be a suitable animal for the experiments to be described.

Methods. *Cryptococcus neoformans* was cultured for 2 to 3 days on Sabouraud's glucose agar. The living cells were suspended in 0.85% solution of NaCl and were adjusted to a concentration of 1:200 by volume. Approximately 0.03 ml. of this suspension was injected with a 27 gauge needle into one of the chorioallantoic veins of 9-day old chick embryos after the method of Lee *et al*[5]. Following inoculation, embryos were incubated at 37°C. for at least 2 days in order to permit infection to become established. Incubation of some embryos at 37°C. was continued until they died or hatched. These served as positive controls. After intervals varying from 2 to 5 days others which had so far survived the experiment were removed to a second constant temperature incubator which was adjusted for different experiments at 39, 40, 41, and 42°C., respectively, and were incubated until death or hatching occurred. Still others which were not inoculated were at first incubated at 37°C. for 2 or more days and were then removed to the second incubator at the specified temperature together with infected embryos as uninoculated negative controls. This experiment was designed to show differences in mortality rate of infected embryos at varying temperatures above 37°C.

In a second experiment, chick embryos were inoculated with *Cryptococcus neoformans* and incubated according to the plan of the first experiment. In addition to any which died, specific numbers of living embryos were sacrificed daily after incubation at temperatures above 37°C. was begun. At autopsy of these embryos, blocks from the chorioallantoic membrane, liver, spleen, kidney and lung were fixed in 10% formalin, embedded in paraffin and sectioned. Sections were stained routinely with hematoxylin and eosin. These sections were examined for the presence or absence of lesions and organisms. The relative numbers of organisms in the livers of infected embryos was estimated roughly in tissue sections and in India ink preparations at the time of autopsy. The presence of viable organisms in the livers was determined by culture in Sabouraud's glucose broth at autopsy.

By this experiment, it was hoped to learn what relation death of Cryptococci might have on survival rate of embryos and whether the microorganisms would disappear from infected tissues under the conditions specified.

Results. In Table 1 it is evident that at 37°C. only a small fraction of the uninoculated embryos die before hatching but that an almost equally small fraction of infected embryos are able to survive. The period of greatest mortality in the latter group is from the 5th to 7th day after inoculation.

In the case of inoculated embryos incubated first at 37°C. and then removed to a higher environmental temperature, it will be seen that in the group incubated at 39°C. following incubation at 37°C. for 2 days, about three-fourths of the embryos survived, but if the preliminary incubation at 37°C. was prolonged to 4 days slightly less than two-thirds survived. Of uninoculated embryos subjected to the same conditions, more than nine-tenths survived. In a group of infected embryos incubated at 40°C. following 2, 3, 4, and 5 days preliminary incubation at 37°C., the survival ratios were approximately four-fifths, three-

TABLE 1.—INFLUENCE OF INCUBATION TEMPERATURE ON SURVIVAL

Final Incubation Temperature (°C.)	*Days of Prior Incubation at 37 °C.*	*No. of Embryos**	*Embryos Dying on Days After Inoculation (Terminated on 7th Day After Incubation at Higher Temperatures) Days*										*Survival Rate of Infected Embryos at Indicated Temperatures*	*Survival of Uninfected Embryos at Indicated Temperatures*	*P** (S—Significant) (N.S.—Not Significant)*
			3	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*			
													°C.	°C.	
39	2	18	2	0	0	1	0	1	0	—	—	—	14-18 (77·7)	24-26 (95)	0·01 (S)
	4	23	–	–	4	1	0	0	1	0	2	—	15-23 (65·2)		0·01 (S)
40	2	42	0	2	0	1	1	3	0	—	—	—	35-42 (83·3)		0·001 (S)
	3	26	–	4	0	0	2	0	0	1	—	—	19-26 (73·1)	30-34 (92)	0·001 (S)
	4	21	–	–	3	2	0	1	1	0	0	—	14-21 (66·6)		0·01 (S)
	5	18	–	–	—	5	1	1	0	0	2	0	9-18 (50·0)		0·05 (S)
41	3	25	–	3	0	0	2	0	1	1	—	—	18-25 (72·0)	18-23 (80)	0·01 (S)
	4	14	–	–	3	1	1	0	0	1	1	—	7-14 (50·0)		0·10 (N.S.)
42	3	11	–	1	2	1	0	2	1	0	—	—	4-11 (36·6)	5-12 (40)	0·30 (N.S.)
Infected Controls at 37 °C. exclusive of Embryos Dying in First 2 Days		76	6	6	14	23	11	4	5	—	—	—	7-76 (9·2)	82-84 (98·0)	

* This number represents the embryos surviving up to the time they were placed at the higher temperature. It is not the number of embryos inoculated.
** P—probability was calculated by the chi square method. The level of significance was taken as = <0.05.

fourths, two-thirds and one-half respectively. Almost 9 of 10 uninoculated controls survived under identical conditions. Likewise when inoculated embryos were incubated at 41°C. following preliminary incubation at 37°C. for 3 and 4 days, the survival ratio was about seven-tenths and one-half respectively. The survival ratio of uninoculated controls, however, was now only four-fifths. At an atmospheric temperature of 42°C. following 3 days incubation at 37°C., the survival ratio of uninoculated controls was only two-fifths, but this ratio was likewise approximated by the inoculated embryos. Considering the fact that an appreciable number of uninoculated embryos fail to survive incubation at the higher specified temperature, the salvage rate of the inoculated embryos at these temperatures becomes more striking when compared with the mortality rate of inoculated embryos maintained at 37°C. The number of infected embryos maintained at 42°C. was so small, however, that the results, though strongly suggestive of protection, were not statistically significant when analyzed by the chi square method.

The results of the second experiment are recorded in Table 2. It will be seen that extensive lesions associated with viable organisms are present in the inoculated control embryos which were incubated continuously at 37° until sacrifice or death. In the case of embryos incubated at higher temperatures lesions were fewer or absent in embryos incubated for 9 or more days at 39°C. than those incubated at this temperature for shorter periods or in the controls. Even at hatching, however, cultures remained positive in this group. At 40°C., cultures showed no growth on and after the 8th day of incubation at the higher temperature. After 2 days of incubation at this tem-

TABLE 2.—INFLUENCE OF TEMPERATURE ON LESIONS AND VIABILITY OF ORGANISMS

Final Incubation Temperature (°C.)	*Days of Prior Incubation at 37°C.*	*No. of Embryos**	*No. of Embryos Sacrificed Daily***	*Presence of Lesions in Tissue: Days of Incubation at High Temperature Days*										*Days at high Temperature to kill organisms*
				3	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	
39	2	26	2	++	+++	+++	++	+++	+	0	+	0	0	Viable until hatching
				+	+++	++	+	++	+	+	+++	+	0	
40	3	31	2	−	++	+	+++	0	+	+	0			8
				−	+	+	++	+	0	0	0			
40	4	17	1	−	−	++	+	+	+	+++	0			8
41	3	20	2	−	+	+	0	+	0	0	0			6
				−	++	+	+	0	0	0	0			
42	3	12	1	−	+	+	0	0	0	0	0			3
Control	Continuous	24	2	++	+++	+++	+++	+++	+++					Viable until death
				+++	++	+++	+++	+++		****				

* Number surviving inoculation and preliminary incubation at 37°C. until time of incubation at higher temperature.
**Starting one day after incubation at higher temperature.
*** As indicated by cultures.
**** Remainder died prior to 8th day.

perature there was generally a striking reduction in the number of organisms seen in the india ink smears and in the sections. The majority of the organisms were evidently killed in the first 48 hours. This corresponds to the *in vitro* results[2]. At an incubation temperature of 41°C., cultures showed no growth by the 6th day of incubation, lesions appeared small in the early days of incubation, and were not found on or after the 8th day of incubation. This trend was more pronounced at 42°C. when cultures were no longer positive on the 3rd day of incubation and only scattered lesions were observed in embryos sacrificed on the 4th and 5th days of incubation.

Discussion. When incubated continuously at 37°C., the 9-day old chick embryo is highly susceptible to infection with *Cryptococcus neoformans* if inoculated intravenously with a heavy suspension of living organisms. Massive lesions were usually produced in many of the organs which usually prove lethal to the embryo within a few days. The organisms are present in great numbers in the infected tissue and are readily seen in hematoxylin-eosin sections as yeast-like cells surrounded by wide clear spaces representing the presence of capsules. Inflammatory reaction is minimal. Of the internal organs, the liver is most constantly involved and is usually the most heavily infected. Grossly, the liver generally shows whitish irregular plaques on about the 5th day after inoculation. A description of the pathologic lesions will be reported elsewhere.

Since chick embryos show little if any resistance to infection with *Cryptococcus neoformans* at 37°C., and since most can survive incubation at temperatures up to 42°C. with proportionate rises in body temperature, the embryos are adaptable to experiments designed to show the effects of incubation at temperatures above 37°C. on the survival of inoculated Cryptococci. When inoculated embryos were first incubated at 37°C. for 2 or more days to insure infection and then were incubated at 39, 40, 41, and 42°C., respectively, there was a progressive increase of survival rate as compared with incubated control embryos left at 37°C., especially when the mortality rate of uninoculated embryos subjected to the same temperatures is taken into consideration. Thus at 42°C., although the survival rate of uninoculated embryos was only 5 of 12, that of inoculated embryos was 4 of 11. Although the number of embryos was limited, there is the suggestion therefore that at this temperature Cryptococci are destroyed before they in turn can kill the embryo.

In the second experiment, it is interesting that although lesions and viable organisms persisted until hatching in embryos incubated at 39°C., the survival rate was only slightly less than uninoculated controls. Likewise at higher temperatures, the presence of lesions and living Cryptococci were demonstrable for 8, 6, and 3 days at 40, 41, and 42°C., respectively. It is probable, therefore, that at 39°C. multiplication of the organisms is inhibited by temperature of incubation, but that they are not actually killed. At temperatures higher than this, however, the organisms are eventually killed and the tissue lesions become resolved.

From the evidence presented, it may be concluded that *C. neoformans* cells are destroyed in the chick embryo incubated at 40 to 42°C. This observation confirms previous reports that this temperature range is lethal for *C. neoformans* both *in vivo* and *in vitro*.

The results of these experiments indicate that to cure cryptococcosis in the human, induced hyperpyrexia of about 104°F. for 8 days continuously would be necessary. In most instances, patients could not tolerate such treatment. But it is possible that the human may have greater powers of resistance

than the chick embryo so that favorable results might be obtained at lower temperatures. The use of antibiotics as adjuvants might also prove helpful. Fever therapy, even if inadequate to cure the disease, might aid in bringing about a temporary remission.

Summary. The intravenous infection of chick embryos with *Cryptococcus neoformans* produces almost 100% mortality. Incubation at 39, 40 and 41°C. markedly increases the rate of survival. At 40°C. the organisms are completely destroyed in 8 days.

Fever therapy is a possible therapeutic adjunct in the treatment of human torulosis.

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HEPATIC LESIONS AND DYSFUNCTION ASSOCIATED WITH CHRONIC ULCERATIVE COLITIS*

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Previous clinical and pathologic studies have indicated that disease of the liver occurs not infrequently in association with chronic ulcerative colitis. Logan[11] as early as 1919 reported the finding at necropsy of 10 cases showing infiltration of the liver with fat, and of 1 case showing cirrhosis, among a total of 13 cases. Four cases of chronic ulcerative colitis with associated hepatic insufficiency were reported by Comfort, Bargen and Morlock in 1938[4]. Cain and Cattan[1] noted the occurrence of infiltration of the liver with fat in the terminal stages of the disease and felt it might be significant in influencing the course of chronic ulcerative colitis. Tumen, Monaghan and Jobb[14] reported 5 cases of cirrhosis in 151 cases of chronic ulcerative colitis. They felt that factors of malnutrition, long periods of nutritional deficiences, toxemia as often seen in chronic ulcerative colitis, and absorption of toxic materials from the bowel were of possible importance in the development of the cirrhosis. Ross and Swarts[13] reported that 14 of 27 necropsies were considered to show evidence of damage to the liver including cirrhosis, toxic cirrhosis and infiltration with fat.

Four instances of cirrhosis were found by Pollard and Block[12] among 87 cases of chronic ulcerative colitis. In 12 of 27 cases in which they performed sulfobromophthalein tests there was 10% or more dye retention. Warren and Sommers[15] found infiltration of the liver with fat in 33 of 60 necropsies. In 6 of the 60 necropsies cirrhosis of the liver was present.

Our study was conducted to determine the incidence and types of pathologic changes seen in the liver in chronic ulcerative colitis and to assay the possible significance of these lesions clinically.

Material and Methods. Postmortem examination was performed in 91 cases of chronic ulcerative colitis encountered at the Mayo Clinic from January 1, 1936, through September, 1949. The clinical records in these cases were studied and specimens of liver obtained at necropsy were examined grossly and histologically. As a control, the liver was also examined in 90 consecutive necropsies, excluding infants and children and cases in which there was any type of colitis or enteritis. Because approximately half of the cases of chronic ulcerative colitis presented

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varying degrees of peritonitis at the time of necropsy, the liver was examined in another series of 57 cases representing various disease entities exclusive of enteritis or colitis and all presenting evidence of peritonitis at necropsy.

Results. In the 91 cases of chronic ulcerative colitis, the most common finding in the liver was that of infiltration with fat (Table 1, Fig. 1). In 31 cases, there was no appreciable infiltration with fat, and in 13 cases there was mild, probably insignificant change. However, in 14 cases there was moderate infiltration (Fig. 1*a*), and in 33 cases severe infiltration (Fig. 1*b*). The frequency of this finding may be contrasted with the incidence of infiltration with fat in the control series as seen in Table 2.

Infiltration with fat was encountered most frequently in the cases of severe fulminating chronic colitis. Aside from

TABLE 1.—PATHOLOGIC CHANGES IN THE LIVER IN CHRONIC ULCERATIVE COLITIS: 91 CASES

Changes	*Cases*
Infiltration with fat	
Mild	13
Moderate	14
Severe	33
Cirrhosis	3
Abscess	1
Necrosis (center of lobules)	1
Hepatitis	1
Fibrosis of portal triads alone	
Mild	2
Moderate in focal area	1
Cellular reaction of portal triads alone	
Moderate	4
Severe	1
Pericholangitis	1
Amyloid deposits in blood vessels	1
Metastatic carcinoma	2
Intrahepatic thrombosis	5

TABLE 2.—INFILTRATION OF THE LIVER WITH FAT IN CHRONIC ULCERATIVE COLITIS COMPARED WITH CONTROL GROUPS

	Grade of infiltration								
	0		*Mild*		*Moderate*		*Severe*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*Total*
Chronic ulcerative colitis	31	34	13	14	14	15	33	37	91
Control group	72	80	11	12	4	5	3	3	90
Peritonitis control group	39	68	11	19	5	9	2	4	57

TABLE 3.—RELATIONSHIP OF HYPOPROTEINEMIA AND INFILTRATION OF THE LIVER WITH FAT IN CHRONIC ULCERATIVE COLITIS

Total proteins, gm. per 100 cc. serum	*Grade of infiltration*				
	0	*Mild*	*Moderate*	*Severe*	*Total*
6.0 or more	8	1	1	1	11
5.0–5.9	5	2	4	3	14
4.0–4.9	5	2	3	9	19
3.9 or less	0	0	1	8	9

this there was no correlation between the incidence and degree of such change and the duration of the illness. Infiltration with fat seemed to occur most frequently in patients who were severely debilitated by the disease. In general the patients with severe hypoproteinemia had moderate or severe infiltration of the liver with fat. This relationship may be seen in Table 3.

There were 3 cases of cirrhosis of the liver among the 91 cases of chronic ulcerative colitis. In 2 of these the patients were men aged 25 years and 41

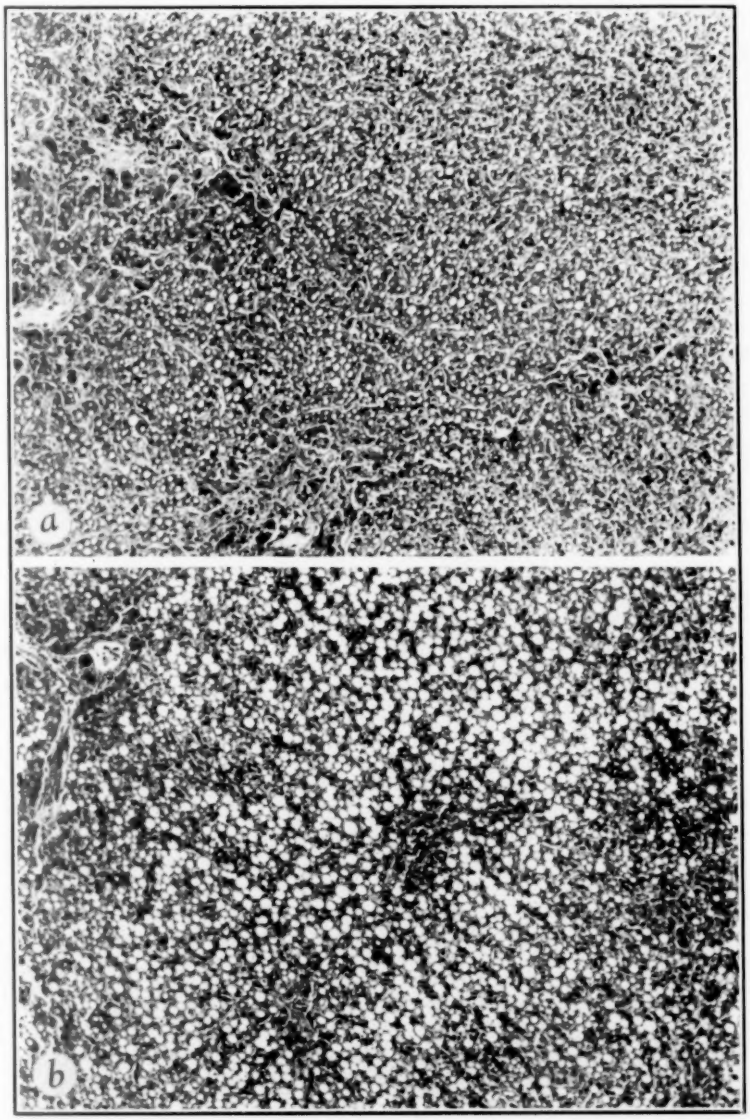

FIG. 1.—Infiltration of the liver with fat in chronic ulcerative colitis. *a*. Moderate degree of infiltration (hematoxylin and eosin x85). *b*. Severe degree of infiltration (hematoxylin and eosin x65).

years, respectively, at death. Both had had long debilitating courses of colitis with many fairly severe exacerbations. One had enterocutaneous and anal fistulas. In both cases, at necropsy the liver grossly was finely nodular, and histologically showed bands of connective tissue which separated regenerated lobules (Fig. 2*a*). The livers weighed 1,616 and 2,712 gm. respectively. The 3rd case was that of a woman aged 34 years who presented a severe fulminating course of colitis of 7 months' duration. Marked ascites and edema de-

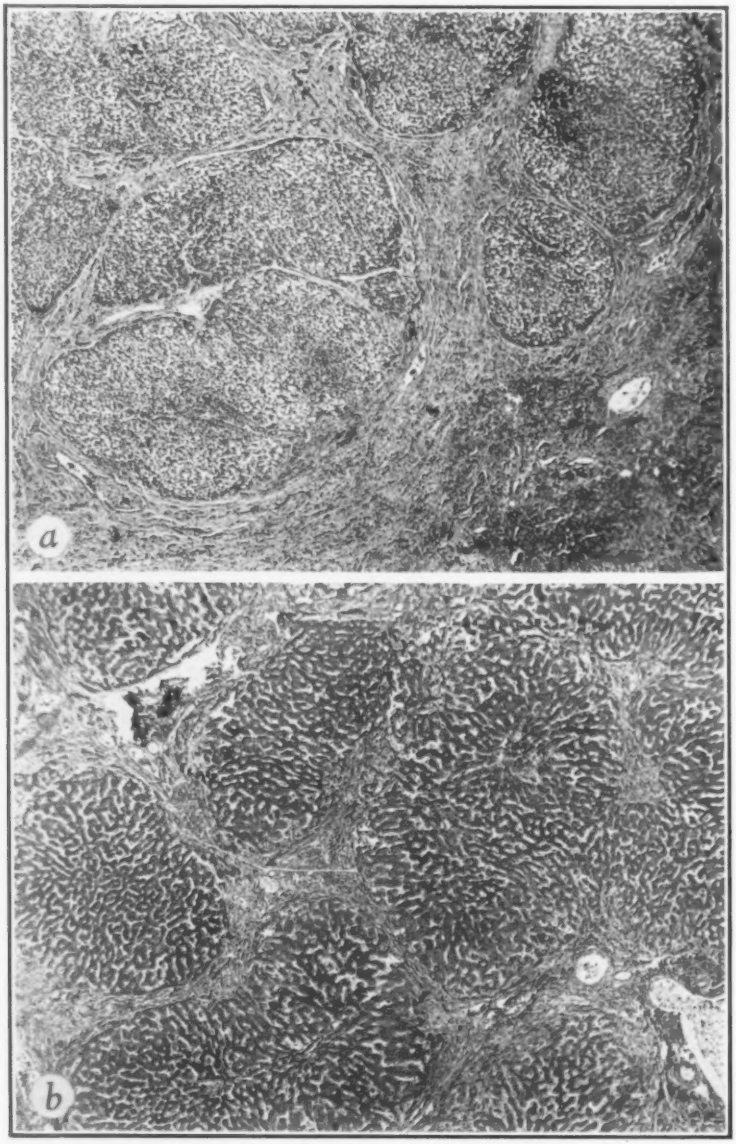

FIG. 2.—Hepatic lesions in chronic ulcerative-colitis. *a*. Cirrhosis of the liver (hematoxylin and eosin x35). *b*. Periportal fibrosis of left lobe of liver (hematoxylin and eosin x35).

veloped 3 months before death. At necropsy the liver grossly was roughly nodular, and histologically showed extensive cirrhosis. It weighed 1,440 gm. It is difficult to be certain in this case that the cirrhosis did not precede the colitis.

In 1 case a large localized portion of the left lobe of the liver showed periportal fibrosis without conclusive evidence of nodular regeneration of the hepatic lobules (Fig. 2*b*). The central veins were somewhat eccentrically placed in some instances, but otherwise

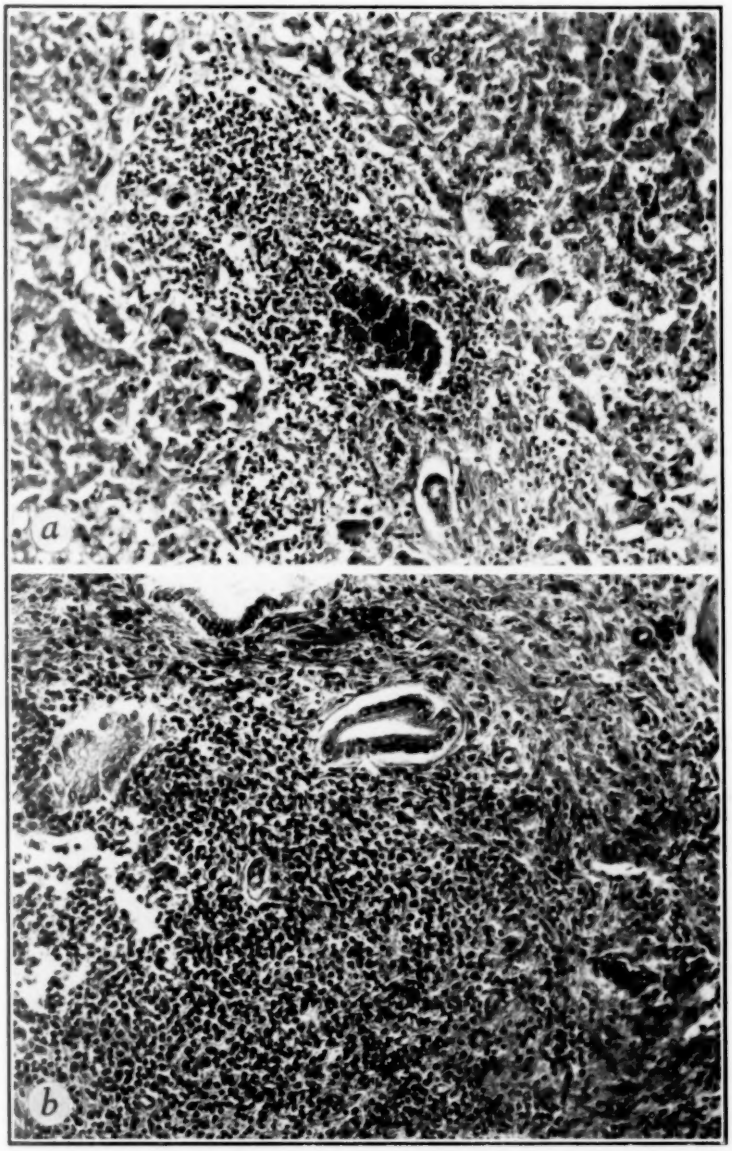

FIG. 3.—Hepatic lesions in chronic ulcerative colitis. *a*. Increased numbers of lymphocytes and plasma cells in periportal spaces (hematoxylin and eosin x155). *b*. Suppurative reaction about bile ducts (hematoxylin and eosin x185).

the lobules appeared normal. This lesion occurred in a man whose colitis had run a long mild course and who had died after cholecystectomy was performed because of nonfunctioning gallbladder which did not contain stones. In 2 other cases there was mild, questionably significant increase of the periportal connective tissue. In 5 cases there was a cellular reaction in the portal triads that was characterized by an increase in number of plasma cells and lymphocytes (Fig. 3*a*). This cellular reaction was moderate in 4 cases and severe in 1. In still another case a pronounced degree of pericholangitis was present with infiltration of polymorphonuclear leukocytes about the bile ducts in the liver (Fig. 3*b*). Necrosis of the hepatic cells in the centrolobular region occurred in 1 case, while in another there was extensive necrosis of cells in the centrolobular region along with infiltration of polymorphonuclear leukocytes throughout the liver.

In 5 instances multiple thrombi were found in the hepatic vessels. In 1 of these an extensive thrombotic process was present, with marked centrolobular necrosis and congestion in the region adiacent to the thrombi. There was only 1 case of pylephlebitis and abscesses of the liver. In this case there had been long-standing chronic ulcerative colitis complicated by the presence of perianal, intra-abdominal fistulas. The hepatic disease in this instance represented a fatal complication.

CLINICAL STUDIES ON THE ASSOCIATION OF HEPATIC DISEASE IN CHRONIC ULCERATIVE COLITIS. Twenty-four patients with chronic ulcerative colitis who entered the hospital for varying reasons were studied with various liver function tests. There was 1 case with hepatomegaly, splenomegaly and jaundice; 1 case of hepatomegaly; 2 cases of splenomegaly alone, and 1 case of marked ascites and mild jaundice secondary to chronic disease of the liver among the group of 24. The patient presenting hepatomegaly, splenomegaly and jaundice entered the hospital primarily because of hematemesis and melena and was found to have esophageal varices with bleeding points demonstrable by esophagoscopy. He was 26 years old and had had severe exacerbations of colitis intermittently over a period of 10 years. The patient presenting ascites and jaundice was a man aged 43 years who had had frequent severe exacerbations of colitis over a period of 6 years, finally necessitating ileostomy and subtotal colectomy 6 years prior to his admission. At the time of this study there was still activity of the colitis in the rectum and the portion of the sigmoid which remained, as ascertained by proctoscopic study. The sulfobromophthalein dye retention at the end of the hour was 34%. The serum bilirubin was elevated, being 1.0 mg. per 100 cc. of serum direct and 0.8 indirect. The cephalin-cholesterol flocculation reaction was 3+. The serum albumin was 1.8 gm. per 100 cc. and the serum globulin was 4.0 gm. per 100 cc.

In 23 instances, sulfobromophthalein tests were performed. In 12 there was grade 0 (0 to 5%) dye retention at the end of 1 hour. In 6 there was grade 1 (6 to 11%) dye retention, and in 4 there was grade 2 (12 to 23%) retention of dye. In one, the patient who had ascites and jaundice, there was grade 3 (34%) dye retention. One patient who had a grade 2 dye retention initially, associated with severe hypoproteinemia, peripheral edema, malnutrition and toxemia, later was found to have a grade 0 retention after improvement of her general condition. In all instances of grade 2 dye retention the patients presented acute exacerbations of the colitis with associated toxemia, anemia, hypoproteinemia and nutritional deficiencies, except for the patient with ascites and jaundice mentioned previously. Thymol turbidity, Bodansky alkaline phosphatase, and serum bilirubin determinations were essentially normal in all instances ex-

cept for the 2 patients with definite clinical hepatic disease mentioned previously.

Comment. The occurrence of hepatic injury during the course of chronic ulcerative colitis has been ascribed by many observers to factors of malnutrition and toxemia. The supposition that malnutrition is of importance in the evolution of these lesions has been supported by a number of experimental studies. György and Goldblatt[8], and others, have produced infiltration with fat, necrosis and increased periportal fibrosis in rats by means of diets deficient in vitamin B. The production of infiltration of the liver of the rat with fat has been accomplished by diets low in protein although supplemented by choline.[10] Chaikoff and associates[2,3] have reported the development of infiltration of the liver with fat followed by cirrhosis in depancreatized dogs maintained on insulin and in dogs given a diet high in fat. Others[7] have produced infiltration with fat and diffuse hepatic fibrosis in rats by feeding diets high in fat content. Hartroft[9] has noted a rapid development of infiltration of the liver with fat in rats fed deficient diets and felt that subsequently there was a proliferation of connective tissue fibers from the adventitial sheaths of the central veins. The occurrence of hypoproteinemia in many of our patients may be indicative of severe malnutrition. The question whether the hypoproteinemia resulted from the malnutrition and toxemia associated with the colitis or whether it is an indication of hepatic damage is difficult to decide from the available data on these cases. In our opinion the hypoproteinemia present in these patients was usually related to the colitis rather than to the infiltration of the liver with fat. One cannot, however, completely exclude the role played by hepatic dysfunction in the production of hypoproteinemia.

Because Connor[5,6] and others have held that infiltration of the liver with fat may eventually culminate in cirrhosis we were particularly interested in the occurrence of cirrhosis in our cases. Although infiltration with fat was common, frank cirrhosis was found in only 3 of 91 cases of chronic ulcerative colitis. This incidence is no higher than that observed in routine necropsies at the Mayo Clinic. This, of course, does not preclude the possibility that chronic ulcerative colitis predisposes to the development of cirrhosis. This possibility should be seriously considered, inasmuch as it is generally recognized that a fatty liver is more vulnerable to various injuries than is a normal liver. The occasional occurrence of necrosis of the hepatic parenchyma and of inflammatory reaction and fibrosis in the periportal spaces may be indications of this increased vulnerability. The question whether these lesions or infiltration with fat can be considered as a precursor of cirrhosis could not, however, be determined from this study.

The clinical studies of hepatic function in the course of chronic ulcerative colitis indicated that abnormalities occurred frequently during exacerbations of the disease. When the colitis subsided the hepatic function usually returned to normal, indicating that anatomic lesions, if they occurred, were probably reversible.

Summary. Infiltration of the liver with fat is frequently observed in chronic ulcerative colitis at the time of necropsy. It occurred in moderate to severe degree in 47 of 91 cases (52%) in our series. It is probably related to the malnutrition, toxemia and debilitating effects of chronic ulcerative colitis. Cirrhosis was found in 3 of the 91 necropsies. Although this incidence is no higher than that observed in routine necropsies, the possibilities that chronic ulcerative colitis predisposes at times to the development of cirrhosis should be seriously considered. Necrosis of hepatic parenchyma, inflammatory reactions and fibrosis in

the portal regions and intrahepatic thrombosis were occasionally observed.

Clinical studies indicated frequent abnormalities of liver function reactions in cases of severe chronic ulcerative colitis. These abnormalities of function often seem to be of a temporary nature. Occasionally, however, studies of function may uncover cases of severe chronic hepatic disease during the course of chronic ulcerative colitis.

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PATHOLOGICAL LIVER WITH MINIMAL OR NO CHANGE IN "LIVER TESTS"

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The purpose of the present study is to analyze various conditions of histologically proved liver disease, in which "liver tests" disclosed minimal or no abnormality. The cases studied were spotted in a routine examination made in patients with liver disease or suspected liver disease during the last 5 years (1945-50).

Methods. The following biochemical determinations were performed simultaneously: direct and total bilirubin; total cholesterol and cholesterol-esters; serum alkaline phosphatase; plasma proteins, albumin and globulin; prothrombin time; intravenous hippuric acid; thymol turbidity; cephalin-cholesterol flocculation; quantitative per diem urinary and fecal urobilinogen and bromsulfalein retention (the last obtained 30 minutes after injection of 5 mg. of dye per kg. body weight; the results are expressed in per cent, a concentration of 4 mg. % representing 100%). The techniques of these different tests have previously been stated[2]. Liver biopsies were obtained in all cases, either at laparotomy or by using the Vim-Silverman needle.

Results. The liver tests were found unaltered or very slightly altered in the following conditions:

Metastatic cancer. The study included 25 individuals with an enlarged nodular liver which proved to be riddled with metastases from various organs. The origin of metastasis was: adenocarcinoma of pancreas 9, colon 6, stomach 4, jejunum 1, retinoblastoma 1, adenocarcinoma of undetermined origin 1, and squamous cell carcinoma of the esophagus. Splenomegaly was absent in all cases and jaundice was present in only 5. The various tests showed a number of similarities. The cephalin-cholesterol flocculation, the thymol turbidity, prothrombin time and the urinary urobilinogen were within normal range in all. In only one case was there a lowering of the cholesterol-cholesterol ester ratio below 60, and in 3 the hippuric acid elimination was below 0.7 gm. In these 3, however, renal function was not tested. Four cases disclosed elevation of the total serum cholesterol above 300 mg. per 100 cc., the highest being 580 mg. Elevation of alkaline phosphatase above 4.4 units occurred in 24 of the 25 patients, and above 10 units in 17. Abnormal bromsulfalein retention above 10 was found in 15 of the 20 cases without jaundice. It was not tested in jaundiced cases. Figure 1-A illustrates a microphotograph of a squamous cell carcinoma of the esophagus with all tests within normal range.

Primary hepatoma. Two cases of primary hepatoma of the liver were studied. The first, a 33-year-old non-jaundiced male, had had a slow growing hepatic tumor for 8 years which had produced an enormous nodular enlargement. The only abnormal tests detected were elevation of alkaline phosphatase to 27 units and a bromsulfalein retention of 15. The second case, a 63-year-old Chinese with a considerably enlarged and nodular liver, with jaundice, whose micro-

photograph is illustrated in Fig. 1-B, had a slight elevation of total cholesterol, 360 mg. per 100 cc. with 235 esters and an alkaline phosphatase of 13.5 units. All other tests in these 2 individuals were within normal range.

Other focal non-tumorous conditions. Four cases were studied with focal non-tumorous conditions in the liver. The first was a non-jaundiced, 67-year-old female with a very hard and bulging non-tender liver, without splenomegaly. Roentgen examination disclosed a typical fist-sized calcified echinococcus cyst of the liver. The only abnormal tests were an alkaline phosphatase slightly elevated to 6.7 units and bromsulfalein retention of 22. The second patient with marked hepatosplenomegaly, proved histologically to be a follicular lymphoma, is illustrated in Fig. 2-A. The only abnormal test was a slight elevation of alkaline phosphatase to 5.7 units. The third case with histoplasmosis and multiple granulomas in the liver, as illustrated in Fig. 2-B, also had normal tests. The last case with sarcoidosis had hepatosplenomegaly. The typical histological changes of the disease in the liver were found as illustrated in Fig. 2-C. All tests in this patient were within normal range.

Conditions associated with liver scar-

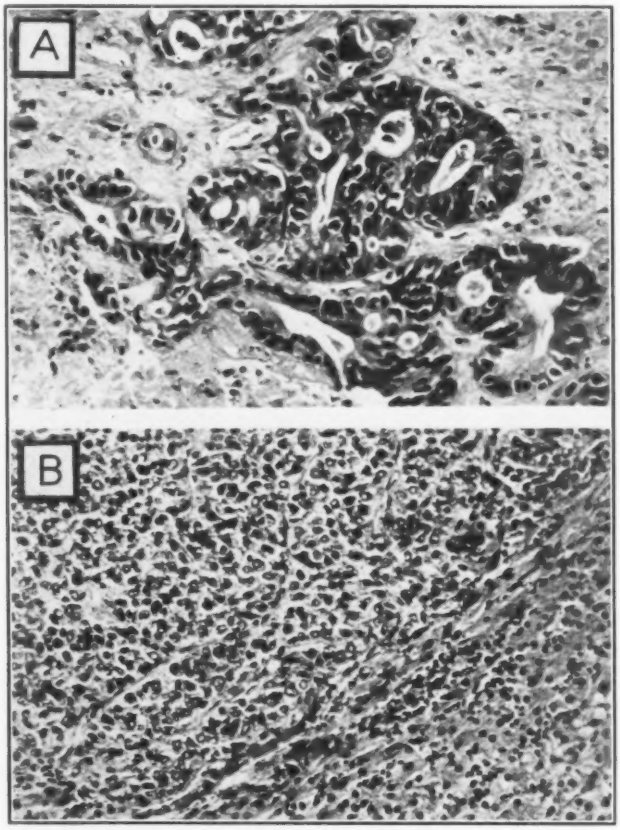

FIG. 1.—Punch liver biopsies in metastatic and primary cancer without alteration of tests of parenchymal function. A. Liver metastasis from squamous cell carcinoma of the esophagus. H and E stain (× 150). B. Hepatoma of the liver. H and E stain (× 207).

ring. In a previous study[3] of 20 asymptomatic cases of portal cirrhosis the value of the tests expressing parenchymal function were found inconstant and unreliable, indicating that fibrosis of the liver itself does not alter parenchymal function. Bromsulfalein tests turned out to be the best screening test and were abnormal in 65% of the cases. Increased thymol turbidity and cepha-

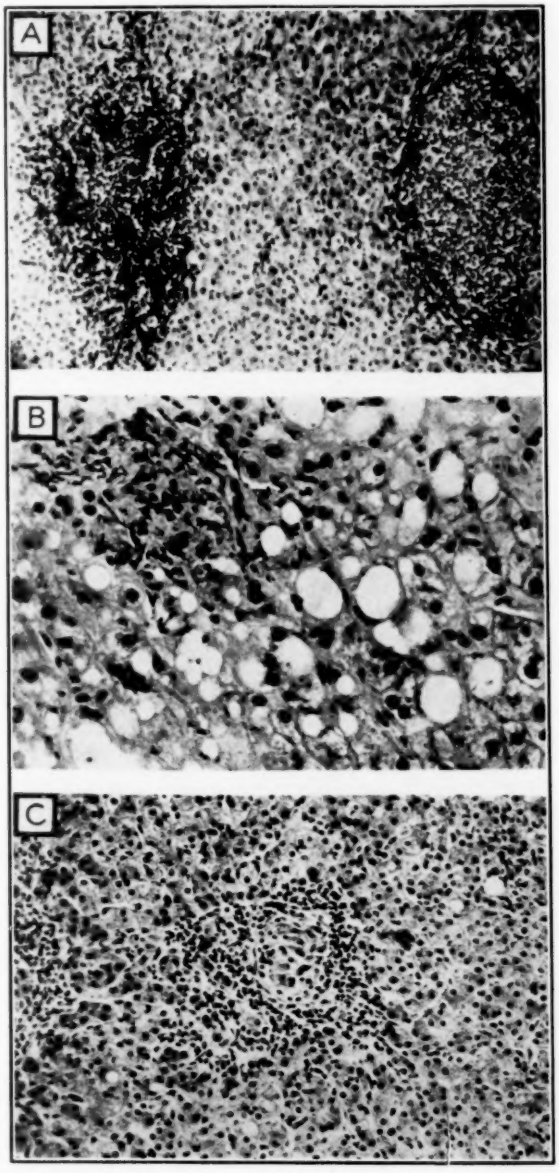

FIG. 2.—Punch liver biopsies in focal non-tumorous conditions involving the liver without altering parenchymal function. A. Giant follicular lymphoma. Hematoxylin, eosin and azure stain (× 75). B. Granuloma and mild parenchymal changes in histoplasmosis. H and E stain (× 200). C. Sarcoidosis. H and E stain (× 100).

lin-cholesterol flocculation occurred in approximately one-third of the cases. This is illustrated in Fig. 3-B in a case of a chronic alcoholic patient with portal cirrhosis. A retention of 15 was the only abnormal test found. In other conditions with diffuse fibrosis but little or no parenchymatous changes, similar findings were observed. Of 2 cases of hemochromatosis studied here,

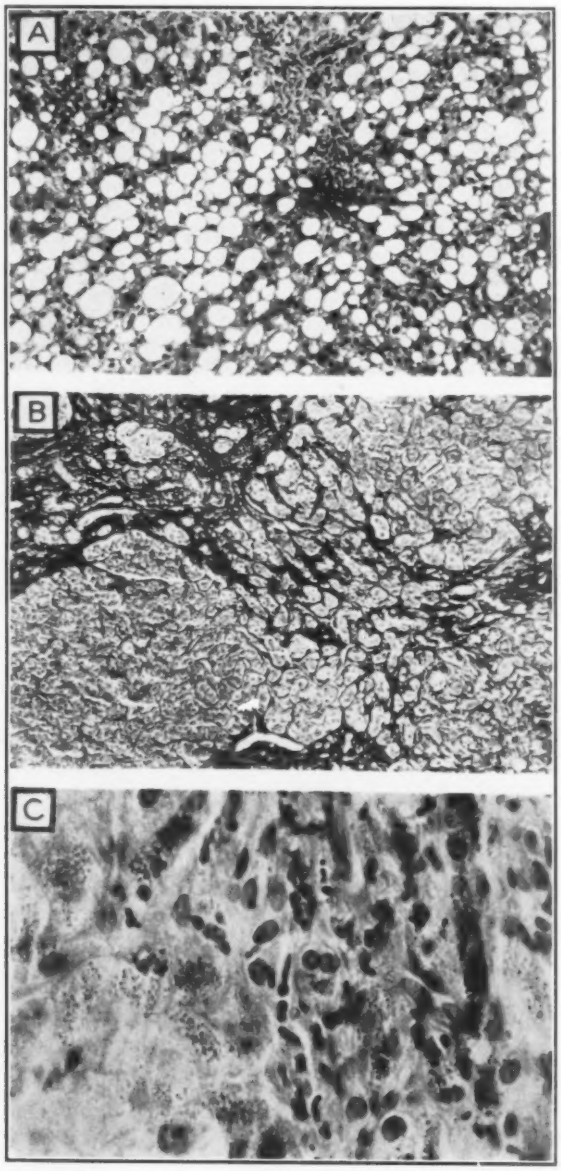

Fig. 3.—Punch liver biopsies in cases with diffuse liver fibrosis with minimal or no impairment of parenchymal function. A. Portal cirrhosis of the liver with fatty infiltration of the parenchyma. H and E stain (× 106). B. Portal cirrhosis of the liver with marked fibrosis. Mallory-Azan stain (× 78). C. Hemochromatosis. H and E stain (× 490).

one gave negative results in all tests and the other, illustrated in Fig. 3-C, disclosed a bromsulfalein retention of 40. Similar negative findings were observed in 2 cases of hepatolenticular degeneration (Wilson's disease) not included here, as no histological study was made.

Storage diseases. Three cases with storage diseases were studied. Of these, 2 had Gaucher's disease involving the liver. In one of these, all tests were within normal range, and in the other slightly jaundiced patient, whose microphotograph of a punch liver biopsy is illustrated in Fig. 4-B, there was marked splenomegaly, hepatomegaly, and marked involvement of the liver, spleen, and bone marrow by the disease. The direct reacting bilirubin was 1.9 and the total 2.5 mg. per 100 cc., a bromsulfalein retention of 48, thymol turbidity 9 units, and cephalin-cholesterol flocculation plus 3. All other tests were normal.

An essential cholesterol storage disease was observed in a 7-year-old boy with marked hepatosplenomegaly. Typical large finely vacuolated cells were observed in the liver, as illustrated in Fig. 4-A, and in the spleen. The only abnormality of the various biochemical determinations was a slight increase of the alkaline phosphatase to 5.2 units.

Degenerative conditions of the liver parenchyma. Fatty infiltration of the liver parenchyma may not affect significantly tests of hepatic function, as has been disclosed previously[3]. In Fig. 3-A microphotographs of a punch liver biopsy are illustrated, indicating marked fatty infiltration and slight periportal fibrosis in a 44-year-old alcoholic male with hepatomegaly but with latent asymptomatic cirrhosis. All tests were within normal range in this case, except a bromsulfalein retention of 38.

In 1 case of diffuse amyloidosis involving the liver, all tests were within normal range.

Discussion. Liver tests within normal range are not synonymous with the absence of liver pathology, a point not sufficiently emphasized. The fact that tests indicating parenchymal function, such as prothrombin time, hippuric acid, and urinary urobilinogen remain normal despite a pathological liver is not at all surprising, since experimental observations have shown the enormous reserve of function of the liver parenchyma. Approximately one-tenth of the normal parenchyma is able to carry on adequate function[1]. This agrees with observations made frequently in cases with large tumors in the liver, in which tests of parenchymal function are found within the normal range. The present study shows that in primary and metastatic cancer, in focal conditions of the liver, such as echinococcus cysts, sarcoid, storage diseases such as Gaucher's, essential cholesterol storage disease, and in fatty or amyloid degeneration of the parenchyma, the tests of parenchymal function may not be significantly altered.

Liver fibrosis with a compensated parenchyma may disclose no significant change in the tests of parenchymal function[3,4]. This supports the idea that cases of acute hepatitis, who develop post-necrotic scarring of the liver, can be diagnosed only by liver biopsy.

The most frequently abnormal test, and thus the best screening test, was found to be the bromsulfalein retention. Elevation of the alkaline phosphatase and the direct reacting bilirubin, as well as the total cholesterol in several cases, in patients with metastasis or focal conditions of the liver without jaundice, is difficult to explain adequately, but they are probably due to regurgitation of bile produced by mechanical action of these focal conditions.

Conclusions. 1. "Liver tests" within normal range may occur despite disease structure of the liver.

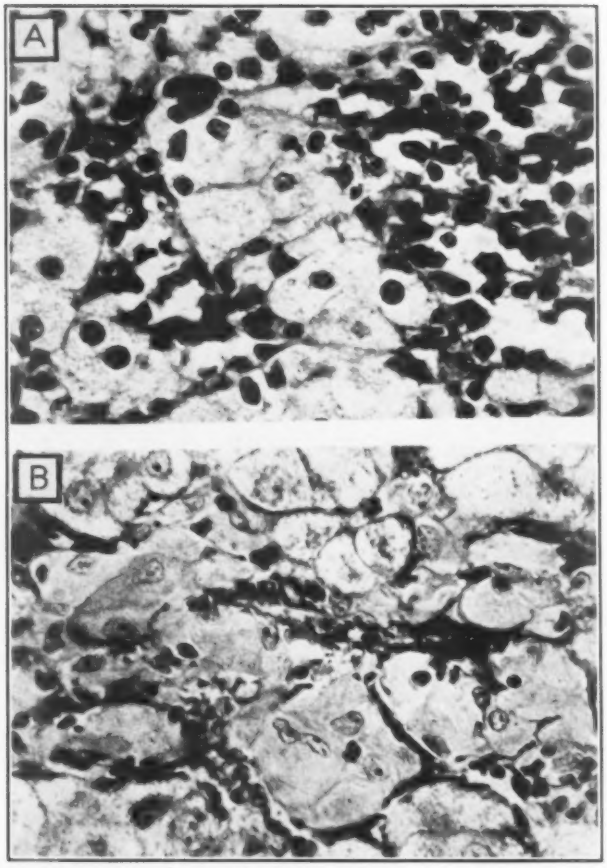

FIG. 4.—Punch liver biopsies in cases with storage diseases involving the liver without producing alteration of parenchymal function. A. Essential cholesterol storage disease. H and E stain (× 720). B. Gaucher's disease. Mallory-Azan stain (× 705).

2. Focal conditions of the liver do not, as a rule, affect parenchymal function.

3. Focal conditions of the liver may be accompanied by increased alkaline phosphatase, total serum cholesterol, and promptly reacting bilirubin in the sera.

4. Storage diseases of the liver, and fatty and amyloid degeneration of the liver, may not affect the present tests used in the differential diagnosis of liver disease.

5. The bromsulfalein test is the best, but not infallible, screening test in the detection of asymptomatic liver disease.

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MYASTHENIA GRAVIS ASSOCIATED WITH HYPERTHYROIDISM: REPORT OF A CASE WITH THYMECTOMY

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Although muscular weakness in patients with hyperthyroidism is common, true myasthenia gravis and hyperthyroidism very seldom occur together[8,9,11].

The case to be reported is interesting because the administration of propylthiouracil apparently cured the thyrotoxicosis, and because dramatic improvement in the myasthenia gravis followed thymectomy.

Case Report: M. L. H., a 44-year old colored female, was admitted to the Detroit Receiving Hospital 8 February, 1948. She gave a history of exophthalmic goiter for 2 years, stating that she had noticed nervousness, excitability, tachycardia, heat intolerance, and weight loss of 30 pounds in spite of a good appetite. Her eyes had been prominent as long as she could remember and she stated that her father's eyes also protruded. She also gave a history of increasing weakness of the extremities for 9 weeks, together with some difficulty in chewing, talking and swallowing. These symptoms had progressed since their onset and the weakness was invariably more marked each day toward evening.

Physical examination revealed an apathetic colored female apparently 50 years of age with temperature 98.4, pulse 100, and respirations 20. Her speech was weak and somewhat slurred. She was unable to raise her arms well and could not lift her head, but would try to move her head into a more comfortable position with the help of her arms. The skin was warm and moist. There was marked bilateral exophthalmos which was greater on the left, and there was divergent strabismus of the right eye. The ophthalmological consultant reported that the right eye measured 23.5 mm. and the left eye 24 mm. on the Hurtel exophthalmometer. Bilateral ptosis was present and the patient was unable adequately to raise her eyelids. She was also unable to close her eyes completely because of the exophthalmos. Marked conjunctivitis with chemosis was present. Moderate diffuse enlargement of the thyroid gland was noted. The chest was clear. Examination of the heart revealed a soft systolic murmur at the apex, but no enlargement. The blood pressure was 130/80. Examination of the extremities showed a fine tremor of the out-stretched hands. There was weakness of all the extremities and great difficulty in performing any coordinated movements. After several feeble efforts to move an extremity the patient would be unable to move it at all.

Initial laboratory work showed the blood count, urine, blood urea, creatinine, chlorides, cholesterol and sugar to be normal. Electrocardiogram showed no evidence of myocardial damage. The tests for syphilis were negative. Glucose tolerance test was within normal limits. The basal metabolic rate on two occasions was +38 and +49%.

Diagnosis. The patient responded dramatically to an injection of 1.5 mg. of prostigmin intramuscularly. This resulted in marked increase in strength which persisted for 4 hours. Biopsy of the calf muscle of the right leg was done and this revealed lymphorrhagia and atrophy of skeletal muscle compatible with myasthenia gravis. On the basis of the above findings a diagnosis of myasthenia gravis was made.

The diagnosis of hyperthyroidism was based on the history, the physical findings (diffusely enlarged thyroid, warm moist skin, fine tremor of the hands, exophthalmos) and the elevated metabolic rate determinations. The accuracy of the latter might be questioned because of the patient's weakness, but the clinical findings were so typical that there seemed no doubt as to the diagnosis of diffuse goiter with hyperthyroidism. Indeed,

all those who saw the patient early in her hospital stay were thoroughly convinced that she suffered from exophthalmic goitre.

COURSE. The patient was given prostigmin and ephedrine. The prostigmin was at first administered orally, but because the patient's response was poor even with rapidly increasing doses, it had to be given intramuscularly. At the same time propylthiouracil was given to control the hyperthyroidism. Desiccated thyroid also was given because of the patient's exophthalmos. The progress and treatment are graphically depicted in Fig. 1.

On large doses of prostigmin the patient's response was only fair. Although she could walk a few steps at times, extreme weakness with inability to rise or move about would occur at other times. The hyperthyroidism was well controlled by propylthiouracil and the exophthalmos increased only slightly. The conjunctivitis and chemosis disappeared and the patient was able to close her eyes. Although there was some improvement in the myasthenia gravis with control of the hyperthyroidism, large frequent doses of prostigmin were still required and the response was only fair. Because of the recent onset of the disease, its severity, and the poor response to medical treatment, it was decided that thymectomy should be done.

OPERATION was performed on September 15, 1948. Good anesthesia was obtained by means of endotracheal cyclopropane and intravenous procaine. The sternum was split from the jugular notch to the 4th right interspace and, using an extrapleural approach, the thymus gland was removed in its entirety. The thyroid gland looked and felt normal. It was biopsied. Closure was performed without drainage.

The patient did very well during and immediately following operation at which time she was given 2 mg. of prostigmin intramuscularly every 2 hours and 24 mg.

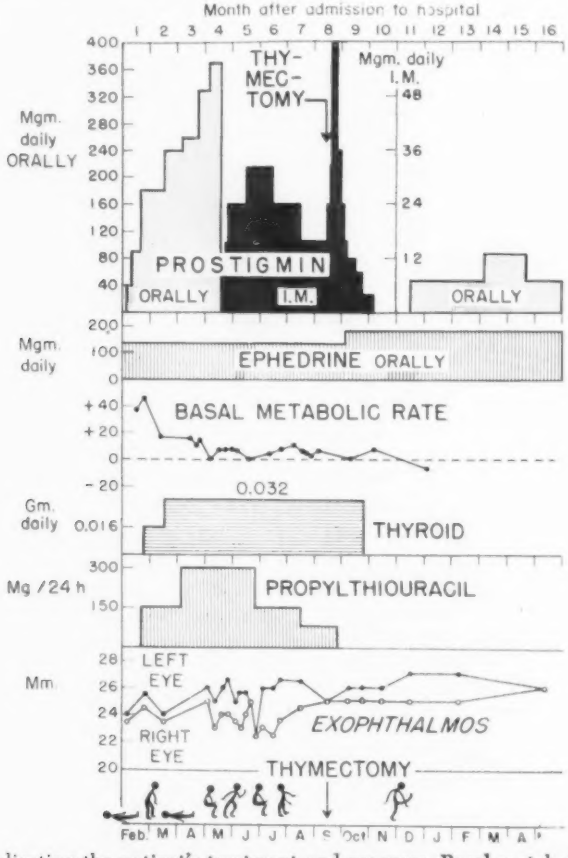

FIG. 1.—Chart indicating the patient's treatment and progress. Basal metabolic rate recorded as percentage of normal. Measurements of the degree of exophthalmos recorded in millimeters by the Hurtel exophthalmometer. Propylthiouracil controlled the hyperthyroidism; thymectomy was followed by dramatic improvement in the symptoms of myasthenia gravis.

of ephedrine every 4 hours. The day after operation, however, extreme dyspnea occurred and was relieved only slightly by bronchoscopic aspiration. The prostigmin was therefore increased until she was receiving 2.5 mg. every hour. Thereupon rapid improvement occurred. Subsequently the dose of prostigmin was cautiously decreased, as shown in the chart, until by the first week of November it was stopped completely.

At this time the patient stated that she felt much better than she had at any time since the onset of her illness. She was able to walk about the hospital, and following discharge on 7 November, 1948, was able to conduct the light activities of necessary household duties without difficulty. Questionable weakness reappeared in December, 1948, and since that time she has been given relatively small doses of prostigmin by mouth and her symptoms have been well controlled. There has been no exacerbation of the myasthenia during a 2-year period of observation.

Propylthiouracil was stopped in the latter part of September, 1948. The basal metabolic rate has remained normal and the patient has had no symptoms of hyperthyroidism. The biopsy of the thyroid gland performed at the time of thymectomy showed only lymphoid hyperplasia but otherwise was normal. The patient's exophthalmos increased slightly by measurement but has not given her any difficulty as far as visual or other symptoms are concerned.

Gross examination of the tissue removed at operation showed an H-shaped thymus gland weighing 35 gm. (Fig. 2). Attached to it was some mediastinal fat, weighing 5 gm. Histological examination of the thymus showed no evidence of tumor. The thymic cells were of two patterns. The Hassal's corpuscle cells were somewhat vacuolated and had an abundance of eosinophilic cytoplasm. The stroma of the organ was composed of cells resembling lymphocytes. These were arranged in some areas in a definite follicular pattern with germinal centers. Much of the gland had been replaced by fat and fibrous tissue.

FIG. 2.—Photograph of the thymus gland. The tissue at the lower right is adherent mediastinal fat. The thymus gland itself weighed 35 gms.

Comment. The treatment of myasthenia gravis is primarily medical. Neostigmine (with or without ephedrine) is the drug of choice[4,10] and recently tetraethylpyrophosphate (TEPP) also has been used successfully[6,10]. When hyperthyroidism and myasthenia gravis occur together it would seem wise to treat the hyperthyroidism first since medical management of the myasthenia then is more likely to succeed.

Unfortunately medical treatment of myasthenia gravis occasionally fails and it is in these cases that thymectomy may be of value. Sometimes at operation a tumor of the thymus is found. If such a tumor can be located and removed improvement in the myasthenia is almost certain to occur. Indeed, it was the excellent result which Blalock obtained following excision of a thymoma in 1936[3] which prompted him to introduce the operation of thymectomy in selected cases of myasthenia gravis even in the absence of tumor[2]. Several groups of cases treated by this operation have been studied and reported since Blalock's original work. At the present time there are some who question the value of the procedure[5], but most of those interested in the problem have reported favorable results, although improvement has been less marked in cases without tumor than when a tumor was found and removed[1,7]. It is admitted that the procedure requires further evaluation, but in our present state of knowledge it would seem reasonable to consider thymectomy for those patients who have the more severe forms of myasthenia gravis and who have had an unsatisfactory response to medical treatment.

The case herein reported tends to support this view. For although the thyrotoxicosis was completely controlled by propylthiouracil, weakness persisted and the patient remained disabled even when given large doses of neostigmine and ephedrine. Thymectomy was followed by such marked and rapid improvement that spontaneous remission seems highly improbable.

Summary. 1. A case of associated hyperthyroidism and myasthenia gravis is presented.

2. The hyperthyroidism was completely controlled by propylthiouracil and has not recurred.

3. Myasthenic symptoms were poorly controlled with doses of neostigmine which totalled 375 mg. per day orally and somewhat better but inadequately controlled with 32 mg. daily intramuscularly.

4. Thymectomy was followed by a marked reduction in neostigmine requirement to 45 mg. orally per day and permitted rehabilitation of the patient.

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TOTAL HYDROLYZABLE CYSTINE IN BLOOD SERUM IN DIABETES*†

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Recent evidence suggests that glutathione may play an important role in normal carbohydrate metabolism[4,7,11]. That insulin may influence the glutathione concentration in blood and tissue has also been demonstrated[8,22]. There is some evidence that low levels of glutathione may occur in new untreated cases of diabetes[19]. In view of these data pertaining to glutathione, it seemed of interest to determine whether normal individuals and diabetics exhibited any differences in blood levels of 2 amino acids closely related to glutathione; namely, cystine and cysteine.

In the free state neither cystine nor cysteine occurs in the blood serum to any appreciable extent. Both of these amino acids are present as integral parts of the serum protein. They are determined, therefore, after hydrolysis of the protein (or more simply of the whole serum) and their concentrations, after hydrolysis, interpreted in relation to the serum protein. In this paper evidence is presented indicating that the blood serum of diabetics, taken as a group, tends to show lower values for total hydrolyzable cystine than that of normal individuals. The lowered values in the diabetic person frequently accompany a lowered ratio of cystine to protein, suggesting that the serum protein of the diabetic may deviate from the normal in cystine composition.

Terminology. Throughout this paper, the term total cystine has been used to designate all cystine plus all cysteine present after hydrolysis of the separated serum of the blood. It includes both the cystine (S-S) and the cysteine (S-H) present in the free and combined form in serum. The term S-S (calc.) is a calculated value, the arithmetical difference between the total cystine value and the cysteine value, and has been used to identify the S-S acid, cystine, alone in distinction from the S-H acid, cysteine.

Methods. *Collection of Blood.* For all determinations venous blood, obtained either after an overnight fast or 3 to 4 hours after a meal, was used. Care was exercised in the use of a tourniquet so that no appreciable stasis occurred during the withdrawal of the blood. All cystine, protein and, in certain cases, cholesterol determinations were made on the clear serum separated after coagulation had taken place. Badly hemolyzed serum was discarded. All samples of serum or blood were kept in the refrigerator at about 4°C. until used. Determinations were carried out usually either the day the serum was collected or the day following. In the case of cystine determinations, check hydrolyses made on serum after 2 or 3 days showed no significant variation to occur during this time interval.

Total hydrolyzable cystine and hydrolyzable

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cysteine were determined by the methods of Vassel[20] on hydrolysates of whole serum. (No appreciable difference was found between the values obtained when hydrolysis was applied directly to whole serum and when it was applied to the precipitated protein of the serum.) Our adaptation of the method was as follows:

1) *Hydrolysis. a) For whole serum.* A known quantity, usually 0.4 cc., of clear serum was measured into a 50 cc. round bottomed centrifuge tube equipped with a ground glass top which fitted an 18 inch straight reflux condenser. To the serum 2.5 cc. of formic-hydrochloric acid* were added and the tube immediately fitted to the condenser and placed in an oil bath on an electric plate maintaining a temperature of 118 to 120°C. for 18 hours (usually overnight). In the top of the condenser and extending about two-thirds of the way down was a tube through which nitrogen gas flowed continuously during the 18-hour period. A flow of 2 to 3 liters per minute insures sufficient nitrogen and does not cause evaporation of the hydrolysate. Appreciable evaporation during hydrolysis must be avoided.

b) For the protein precipitate of whole serum. If hydrolysis was applied to the precipitated protein of the serum either 0.4 or 0.5 cc. of serum was used and the protein precipitated by adding 2.5 cc. of water followed by 5 cc. of 10% trichloracetic acid. After centrifugation and draining, 2.5 cc. of HCOOH-HCl were added directly to the precipitate and hydrolysis carried out as in *a*).

c) For the albumin fraction of the serum. Separation of the albumin fraction of the serum was accomplished by precipitation of the globulin with 23% sodium sulfate with the ether modification of Kingsley[9]. The albumin in an aliquot (10 cc.) of filtrate was precipitated with 10% trichloracetic acid. After centrifugation, the supernatant fluid was discarded and 2.5 cc. of HCOOH-HCl mixture pipetted directly onto the precipitate. Hydrolysis of the albumin was then conducted as outlined in *a*).

(2) *Colorimetric determination.* At the completion of hydrolysis the hydrolysate in each case was quantitatively transferred to a 10 cc. glass-stoppered cylinder with 5N HCl and made to a final volume of 10 cc. with the acid. Determinations for total cystine and for cysteine were carried out, according to the Vassel directions, on either 1 cc. or 0.5 cc. aliquots of the hydrolysates obtained from the hydrolysis of the whole serum or from the albumin. The difference between the value obtained on the whole serum and that obtained on the albumin was regarded as globulin cystine. A Leitz photoelectric colorimeter with filter No. 401 was used in the final determination and two standards and a blank were usually run with each set of determinations as slight variations in the standard curve sometimes occurred.

In the use of this procedure for the determination of total cystine and cysteine we have found that: 1, the technique used in hydrolysis is unaffected if the serum contains glucose up to the equivalent of 1000 mg. per 100 cc.; 2, the technique used in hydrolysis is accurate when applied to lipemic blood if a smaller aliquot of serum, usually 0.2 cc., is used with the same (2.5 cc.) quantity of formic-hydrochloric acid; 3, to obtain good reproducible results with the colorimetric procedure the kind of zinc used is extremely important. Mallinckrodt's zinc metal dust has given us the best results. The character of the zinc must be such that nearly complete solution occurs during the 45 minute period at room temperature. Either instantaneous solution or insufficient action at this point may introduce error.

Total protein, albumin and globulin. In all protein determinations a micro-Kjeldahl procedure with Nesslerization was used. All determinations were done in duplicate. Many were done in triplicate and quadruplicate, using as an additional check a slight modification of the Weichselbaum method[21] for serum protein determination. Results usually checked within 1%. Fractionation of the serum was accomplished with 23% Na_2SO_4. Albumin was determined both by micro-Kjeldahl and a modified biuret method on the sodium sulfate filtrate. Globulin was calculated as the difference between total protein and albumin.

Total cholesterol was determined by the Bloor[2] method and was done merely as an index of the fat content of the blood of the diabetic patients.

Subjects. The data reported in this paper are based on a study of 25 normal and 52 diabetic individuals. The normal persons, 10 males and 15 females, ranged in age from 20 to 45 years. The diabetic patients, 32 males and 20 females, were from 14 to 68 years of age. The duration of diabetes varied from less than 1 month to 25 years and in 25 of the 52 patients had been present for 5 years or longer. In 14 cases the duration of diabetes was 1 year or less. With the exception of 6 cases all patients had been taking insulin for at least 1 month, and at the time of the study were receiving from 4 to 84 units daily. Approximately half

* 58 cc. concentrated hydrochloric acid and 63 cc. of formic acid[12].

of the patients were ambulatory and their condition under reasonably good control as indicated by tests of the blood and urine for sugar.

In the group of diabetics studied, only those cases have been included which were essentially free of complications. None had any infection at the time of study; 3 cases only had any appreciable (more than 20 mg. per 100 cc.) albumin in the urine, and these were not included in the compilation of the protein data. Although there are obvious disadvantages in considering together individuals with diabetes of diverse character, the data at hand seem insufficient to warrant further classification.

In addition to the normals and diabetics studied, a few values obtained on patients with diseases or conditions other than, or in addition to, diabetes have been included.

Results. Comparison between the total hydrolyzable cystine of normal individuals and of diabetics has been made by use of distribution graphs (Figs. 1, 2, 3, 4). The values obtained both for total cystine and for the serum proteins have been grouped in definite ranges and each range plotted against the per cent of cases of normals and of diabetics falling in that range. The cystine-protein ratio has been expressed as $\frac{\text{total cystine in mg. per 100 cc.}}{\text{total protein in mg. per 100 cc.}} \times 100$ to facilitate ease of comparison.

Total cystine in serum. The data in Fig. 1 indicate that the total cystine of serum of 76% of the normal persons lay in the range of 325 to 374 mg. per 100 cc. (40%, 325 to 349 mg. and 36%, 350 to 374 mg.) This range, therefore (325 to 374 mg. per 100 cc.), has been used as the expected range for normal persons. In comparison with these figures, the diabetics showed a definite decrease in the total cystine of serum. Only 14% were within the above range (8%, 325 to 349 mg., and 6%, 350 to 374 mg.), while 69% gave results below 325 mg. %.

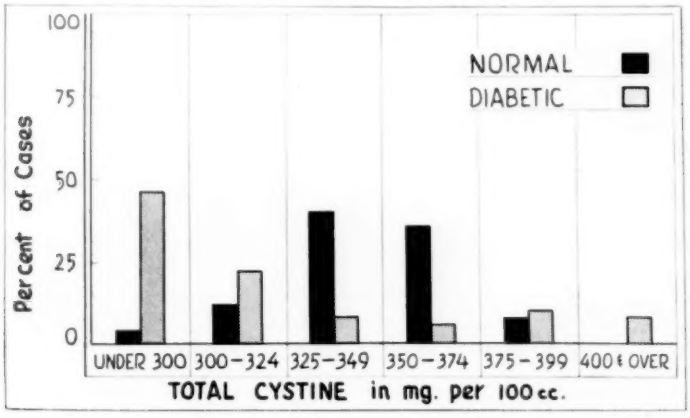

FIG. 1.—Total hydrolyzable cystine content of the blood serum of normal and diabetic individuals with values arranged as to percentage of cases falling within certain ranges.

Similar decreases in the diabetics were observed in the total cystine of the albumin fraction of the serum protein and to a lesser extent in the residual globulin-containing fraction (Fig. 2).

Together with the tendency in the diabetic for a decreased total cystine in the serum and in the major protein fractions, in each case the values showed also a wider variation from the mean than that found in the normal

group. Thus, the range for total cystine in serum in the diabetic group was 213 to 420 mg. per 100 cc. with a standard deviation of ±46.5 as opposed to 295 to 380 in the normal group and a standard deviation of ±21.5.

Serum total protein and cystine-protein ratios. As shown in Fig. 3a, the distribution of total protein values did not differ greatly in the normal and diabetic groups. This fact indicates that the observed lowered total cystine in

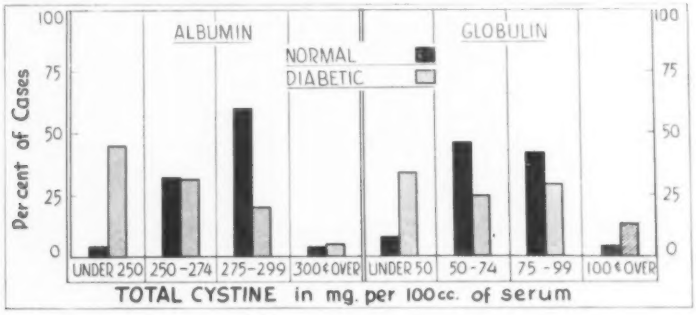

FIG. 2.—Total hydrolyzable cystine content of the albumin and globulin fractions of the serum protein of normal and diabetic individuals. The values are arranged as to percentage of cases falling within certain ranges.

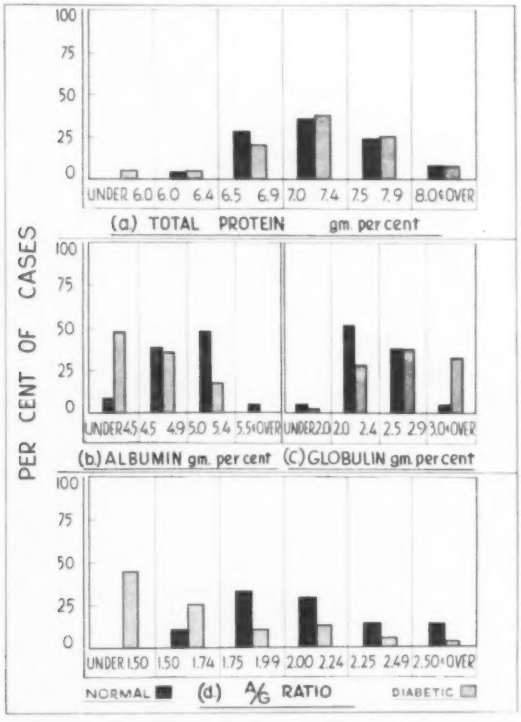

FIG. 3.—Comparison of total protein, albumin and globulin values and A/G ratios in the serum of normal and diabetic individuals.

the serum of the diabetic individual was not merely a reflection of a lowered protein content. It is obvious that a decrease in total cystine with total protein essentially unchanged from the normal would create in the diabetic a cystine-protein ratio below that found in the normal individual.

globulin fractions. Although in the comparison of the normal and diabetic groups there was evidence of little variation in the serum *total protein*, this agreement was not carried over to the albumin and globulin fractions, Fig. 3 (b) and (c). Here a definite trend in the diabetic for a decreased albumin

TABLE 1.—CYSTINE-PROTEIN RATIOS AT VARIOUS LEVELS OF SERUM PROTEIN IN NORMAL AND DIABETIC INDIVIDUALS

	Normals			*Diabetics*		
Total Protein %	*No. of cases*	*Cystine-Protein Ratio*		*No. of cases*	*Cystine-Protein Ratio*	
		Range	*Average*		*Range*	*Average*
Under 6.5	1	4.9	4.9	6	4.1–4.7	4.4
6.5–7.5	16	4.2–5.2	4.8	27	3.9–5.1	4.4
Over 7.5	8	4.5–4.9	4.7	19	3.4–5.3	4.3
	Total range:	Normals 4.2–5.2		Grand average:	Normals	4.79
		Diabetics 3.4–5.3			Diabetics	4.36

TABLE 2.—PERCENTAGE OF ALBUMIN AND GLOBULIN IN TOTAL PROTEIN IN BLOOD SERUM IN NORMAL AND DIABETIC PERSONS

		Albumin		*Globulin*	
Classification	*No. of cases*	(*All values expressed as % of Total Protein*)			
		Range	*Average*	*Range*	*Average*
Diabetics (fasting) series (*a*)	17	51.3–71.2	60.1	28.8–48.7	39.9
Diabetics (fasting) series (*b*)	30	50.7–71.5	61.7	28.5–49.5	38.4
Normals (fasting)	19	61.8–73.4	67.0	26.6–38.2	33.0
Normals Electrophoretic determinations Taylor and Keys[17]	8	61.9–73.3	65.9	26.7–38.1	34.1

No cases included in which an inverse A/G ratio occurred.

This is evident in Table 1, where it will be seen that in every protein range the average cystine-protein ratio for the diabetics was found to be slightly less than that for the normals in the same range. The grand average for the diabetic group was 4.36 (range 3.4 to 5.3) as opposed to 4.79 (range 4.2 to 5.2) for the normal group. Tuchman and Reiner[18] determined the average ratio of cystine to protein in 15 miscellaneous cases to be 4.82.

Serum albumin and globulin and the total cystine content of the albumin and

and an increased globulin was observed which led to a lowered A/G ratio in the diabetic group, Fig. 3 (d). The data in Table 2, tabulating actual percentage of albumin and globulin in the total protein of the diabetics (divided here chronologically into 2 series) in comparison with the normal group support this observation. It is apparent that the percentage of albumin in the protein was consistently lower and that of globulin consistently higher in the diabetic than in the normal group. We have included in this table the electro-

phoretic analyses of Taylor and Keys[17] on 8 normal persons. These are in essential agreement both in range and average with our normals.

Analysis of the data as regards the *cystine content* of the *albumin and globulin* indicates in each fraction some tendency for a decreased percentage in the diabetic group as compared with the normal group (Fig. 4). The evidence suggests the presence in some diabetic serum of protein deficient in cystine and would indicate the deficiency more likely to be associated with the globulin fraction. This tendency is indicated also in averages calculated from the data used in Fig. 4. Thus the average found for the per cent of total cystine in the globulin in the diabetic group is 2.34 as compared with 2.87% for the normal group and 2.80% as the normal value given by Brand[3]. In the albumin fraction no appreciable difference is found in the average, 5.47% (S-S calc. acid) in the diabetic group compared with 5.58% for our normals and 5.57 as the average normal value given by Brand[3]. The range, however, in the diabetic group is significantly greater in the diabetic series, 4.24 to 6.60 as compared with 5.30 to 6.00 for the normals. Our data lead us to believe that in many instances the mathematical averaging of results may easily mask trends which may be of physiological significance. The downward trend in cystine values which we have observed is accompanied by marked variability which may be a reflection, to some extent, of the status of the diabetic condition at the time the blood sample was taken.

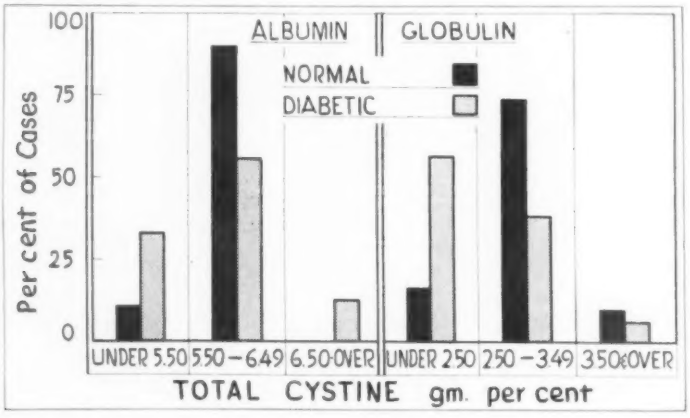

FIG. 4.—Comparison of the total cystine content of serum albumin and globulin in normal and diabetic individuals.

Variability in total cystine in serum of diabetic individuals. In several instances more than one blood sample was taken on the same individual at varying time intervals. In the normal group in 10 different individuals 2 or more blood samples were taken at different times in relation to food during a period of 6 months to 2½ years. A striking constancy was observed in the serum cystine of these individuals with an even greater constancy in the cystine-protein ratio, the 22 analyses on the 10 individuals showing a standard deviation in the cystine-protein ratios of ±0.569, with each individual maintaining an almost constant ratio. In 9 diabetic individuals 2 to 4 samples were taken similarly at different time intervals; in 3 cases 2 samples were taken on the same day, 1 fasting and 1 at 11 A. M. In 4 cases the blood samples

were taken before and after treatment for diabetic coma. In contrast to the constancy observed in the normals, the 20 analyses on these 9 patients exhibited marked variation, showing a standard deviation in the cystine-protein ratios of ±1.535 with significant shifts occurring in some patients during a period of only a few hours. The complete data are given in Table 3. In those diabetics a trend in the diabetic for an actual decrease in the total cystine of the serum with a consequent tendency for a low cystine-protein ratio. The decrease of cystine may occur in either fraction of the serum protein but is more often found in the globulin fraction. Second, there is a definite tendency in the diabetic for an increased serum globulin with a decreased A/G ratio. Third, in

TABLE 3.—TOTAL CYSTINE AND TOTAL PROTEIN IN SERUM AND THE CYSTINE-PROTEIN RATIO AT VARYING TIME INTERVALS IN NORMAL AND DIABETIC PERSONS

			Normal						*Diabetic*			
No.	*Date*	*Time*	*Total Cystine mg./100cc.*	*Total Protein %*	*Cystine-Protein Ratio*	*No.*	*Date*	*Time*	*Total Cystine mg./100cc.*	*Total Protein %*	*Cystine Protein Ratio*
1	12/20/45	10 A.M.	343	—	—	1	12/1/47	F	213	5.2	4.1
	5/26/47	F	338	7.0	4.9		7/17/48	3 P.M.	295	7.0	4.2
	7/10/47	11 A.M.	325	6.6	4.9		8/18/48	F	265	7.1	3.7
							11/20/48	F	320	6.9	4.6
2	6/28/46	F	350	7.0	5.0	2	7/21/48	F	290	7.2	4.0
	5/20/47	F	371	7.5	4.9		7/21/48	11 A.M.	324	7.8	4.1
	12/11/47	F	345	7.1	4.9						
3	12/17/46	10 A.M.	358	7.8	4.6	3	7/30/48	F	310	7.0	4.4
	5/26/47	F	364	7.8	4.7		7/30/48	11 A.M.	300	7.0	4.3
4	5/14/47	F	365	7.9	4.6	4	11/18/48	F	264	6.7	3.9
	4/22/48	F	364	7.8	4.7		11/18/48	11 A.M.	310	6.8	4.6
	5/22/47	F	349	7.2	4.8	5	3/25/48	F	412	8.1	5.1
5	8/30/48	F	340	6.9	4.9		2/27/48	F	412	8.2	5.2
	9/11/48	11 A.M.	340	7.0	4.9						
6	12/1/47	F	323	6.5	5.0	6	Coma	a	220	6.8	3.2
	11/20/48	11 A.M.	320	6.7	4.8			b	210	5.1	4.1
7	3/10/48	F	334	7.2	4.6	7	Coma	a	289	7.2	4.0
	11/17/48	11 A.M.	359	7.5	4.8			b	237	5.6	4.2
8	5/18/48	F	380	7.8	4.9	8	Coma	a	377	8.2	4.3
	10/28/48	11 A.M.	380	7.8	4.9			b	260	5.1	5.1
9	5/18/48	F	360	8.0	4.5	9	Coma	a	391	8.7	4.5
	10/28/48	11 A.M.	370	8.0	4.6			b	277	6.6	4.2
10	5/18/48	F	340	6.7	5.1						
	12/16/48	11 A.M.	335	6.4	5.2						

F Fasting
a Admission sample or very early after beginning of treatment
b Sample obtained approximately 24 hours later

having a fasting and an 11 o'clock sample taken on the same day, an appreciable rise occurred in the percentage of total cystine in the globulin. Thus, data not given in Table 3 indicate that in Case 2 an increase from 1.6% fasting to 2.7 at 11 A. M. was observed, in Case 3 from 2.50 to 2.9, and in Case 4 from 3.3 to 4.9%. Although the data are not extensive, the changes in the diabetic group are striking in comparison with the normal group. Whether this variability and the noted increases in globulin cystine in the 3 cases observed may be related to insulin therapy is problematical, but results of animal work suggest such a possibility.

Discussion. Our data indicate, first, repeated analyses over periods of time, the individual diabetic shows a greater degree of fluctuation than the normal person in serum protein concentration, in total cystine content of the protein and in the cystine-protein ratio.

As regards the cystine content of the blood proteins in diabetes, we have found very few instances in which measurements of this amino acid in the blood of diabetics have been recorded. Lang and Braun[10] reported 1 diabetic case with cystine values for 2 fractions of the serum globulin significantly lower than the corresponding averages for 10 normal persons. Balint *et al*[1] give a normal average of 3.50%, and give 1 case of diabetes as 2.88% in

whom the serum protein was 6.38%. Serum protein changes in diabetes have been reported by several workers. Peters and Eisenman[14] observed slightly decreased albumin and increased globulin in 14 cases where some degree of protein starvation was detected. Lewis, Schneider, and McCullagh[12] in electrophoretic studies reported in diabetic acidosis a low albumin and high β globulin concentration which became normal when the diabetes was controlled. In diabetes complicated with retinitis, these authors[15] observed a persistent tendency for low albumin and increased β globulin.

using this method of fractionation on pathological blood, although it is generally conceded to be satisfactory for normal blood. The criterion as to what constitutes "pathological" blood is difficult. In the group of 40 diabetic patients on whom protein studies were made none had evidence of disease other than diabetes. No blood findings to our knowledge were abnormal except blood sugar, and in 3 cases a somewhat elevated cholesterol. No blood was lipemic. Since glucose up to an equivalent of 1000 mg. per 100 cc. has no effect on the sodium sulfate fractionation as we have used it, there

TABLE 4. TOTAL CYSTINE, TOTAL PROTEIN AND THE CYSTINE-PROTEIN RATIO IN CONDITIONS OTHER THAN OR ACCOMPANYING DIABETES

No.	*Total Cystine* mg. /100cc.	*Total Protein* %	*Cystine-Protein Ratio*	*Condition*
1	360	7.5	4.8	Rheum. ht. dis., compensated
2	345	6.7	5.1	Uveitis, chronic
3	375	7.0	5.4	Sarcoma, larynx
4	280	6.4	4.4	Prostate, benign hypert.
5	367	7.4	5.0	Bronchiectasis
6	175	4.9	3.6	Chronic glomerul. nephr.
7	315	6.9	4.5	Gout
8	282	6.0	4.7	Normal pregnancy (2 mos.)
9	285	5.8	4.9	Normal pregnancy (4 mos.)
10	245	6.0	4.1	Normal pregnancy (7 mos.)
11	255	7.6	3.4	Gout, chronic nephr. with uremia and diabetes
12	270	6.6	4.1	Diabet. pregnancy (2 mos.)
13	220	6.4	3.4	Diabet. pregnancy (4 mos.)
14	213	5.9	3.6	Diabet. pregnancy (7 mos.)

The significance of our observed changes in albumin and globulin in the diabetic may be criticized in view of the use of sodium sulfate as the fractionation agent. Since our primary interest, however, lay in a comparison of the diabetic and the normal in regard to the cystine concentration of the 2 major fractions of the serum protein, its use seemed permissible as it yielded albumin filtrates easily adapted to cystine determinations. Whether or not the method differentiated true albumin or albumin plus some globulin did not seem to us to invalidate the comparison. We are quite aware of the possibilities of erroneous results in would appear no reason for not accepting as valid the protein results obtained in the diabetic group.

Although the observation of increased globulin and lowered albumin is common to many diseases, we are unaware of any extensive data regarding the cystine content of the proteins in these diseases. Faber[6] reported cystine changes in albumin in amyloidosis and Crossley[5] in dogs with acute pneumococcus infection. No comment was made in these cases of the ratio of total cystine to total protein. From the limited data we have of cystine-protein studies in diseases or conditions other than diabetes (Table 4), it may be ob-

served that a lowered total cystine and a lowered cystine-protein ratio may occur in diseases other than diabetes, particularly advanced nephritis. Pregnancy, it would appear, tends to produce lower concentrations, but it will be noted that in the diabetic pregnancies the effect is accentuated.

That changes in serum protein composition in relation to fluctuations of free sulfhydryl groups may be of real significance in several pathological conditions has recently been shown by Schoenbach *et al.*[16]. Our data give additional weight to the observations of these authors and place diabetes in the category of diseases in which there may be variation in the sulfur-containing amino acids of the serum protein. Our data suggest that in the diabetic the serum globulin may be of poorer quality as regards total cystine, even though this fraction of the protein is present in increased quantity. If some of the inherent changes predisposing to abnormal carbohydrate metabolism or to other pathological conditions are concerned with changes in glutathione metabolism and to shifts in the closely related amino acids of the serum proteins, a new approach to some factors in the etiology of diabetes may be revealed.

It has been pointed out that, in comparison with the normal, the diabetic shows considerably more variability in the total cystine of the serum and in its relation to the serum protein. We believe from data to be reported elsewhere that insulin activity and the status of control of the diabetes may be factors in this variability.

Summary. 1. The total hydrolyzable cystine in blood serum in 52 diabetic patients, as compared with that in 25 normal individuals, showed a trend in the diabetic for a decreased total cystine in the whole serum and in both the albumin and globulin fractions of the serum protein. In 76% of the normal persons the total cystine values of the serum were betwen 325 and 375 mg. per 100 cc., whereas only 14% of the diabetics had values within this range and 69% had values below 325 mg. per 100 cc.

2. In all ranges of serum protein concentration the diabetics showed a lower cystine-protein ratio than the normals. The average ratio for the normals was 4.79, for the diabetics 4.36.

3. Using Na_2SO_4 fractionation, 46% of fasting diabetics, clinically free of complications, showed an albumin-globulin ratio under 1.5 whereas none of 19 normals had ratios this low. The low ratio was due to an increase in globulin and a decrease in albumin.

4. The decrease of total cystine in the serum of the diabetic was chiefly associated with the globulin-containing fraction. The average percentage of total cystine in the globulin of the diabetic group was 2.34 as compared with 2.87 in the normal group.

5. In the diabetics there was, in the same individual, a striking variability in serum cystine and serum protein concentrations and in the cystine-protein ratio in contrast to a remarkable constancy in the normal individuals studied. It is suggested that this variability may be related to the activity of insulin and state of control of the diabetes at the time the blood sample was drawn.

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THE BLOOD GLUTATHIONE LEVEL AND ITS RESPONSE TO INSULIN IN DIABETIC AND NON-DIABETIC PATIENTS AND A CASE OF INSULIN RESISTANCE*

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During the past few years interest has been focused on the possible role of glutathione in the pathogenesis of diabetes mellitus. This has been due largely to the discovery of the diabetogenic action of alloxan[5] and the prevention of alloxan diabetes by glutathione and other sulfhydril substances such as cysteine, thioglycollic acid and BAL[10,11]. Lazarow has postulated an attractive theory which states that the beta cells of the islets of Langerhans may contain an insufficient amount of glutathione due to the utilization of their sulphur in the production of insulin which contains a large amount of sulphur (3%) in the form of cystine[12]. As a result, these cells are more susceptible to the destructive action of toxic substances such as alloxan than are other tissues.

Conn and co-workers have supported this concept by reporting the production of hyperglycemia, glycosuria, and concomitant reduction of the blood glutathione during the administration of ACTH to normal people[2]. In a later paper they reported a temporary reversal of the hyperglycemia and glycosuria in response to the intravenous administration of glutathione in patients receiving ACTH[3]. It was postulated that an alloxan type of substance was produced due to the altered purine metabolism, and the glutathione inactivated it with resultant protection of the beta cells of the islets of Langerhans. As yet, there is no proof that this occurs.

The role of glutathione in the etiology of diabetes mellitus assumes practical importance when one considers the great deal of evidence showing that experimental diabetes may be reversible in its early stages. If glutathione were involved according to the above theory, its administration in very early cases might reverse the diabetic process.

Thus the blood glutathione values in diabetes mellitus assume considerable interest. Sub-normal values would lend support to Lazarow's theory. There have been reports in the older literature stating that the blood glutathione is diminished in diabetes mellitus and that insulin elevates it[1,8,9,13,15,17]. Examination of the data presented does not warrant such conclusions, particularly in view of the fact that

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the older methods employed for determining glutathione have been shown to be of questionable accuracy.

We undertook a study of the glutathione blood levels in patients with diabetes mellitus using one of the more accurate methods. A study was also made of the effect of large doses of insulin on the blood glutathione of diabetic and non-diabetic patients. The latter were patients undergoing insulin therapy for schizophrenia. In addition, studies of the blood glutathione in an insulin resistant patient with diabetes mellitus are reported.

All references to glutathione refer to the reduced form.

Methods. The patients were divided into 4 groups.

GROUP 1 consisted of 10 normal hospitalized male patients who were awaiting elective ear, nose, and throat procedures. The operation performed was either a sub-mucous resection of the nasal septum or a rhinoplasty. All determinations were made on fasting blood and prior to surgery.

GROUP 2 consisted of 42 patients with diabetes mellitus from the out-patient clinic. Fasting blood samples were drawn in the morning when the patients reported to the clinic. These were analyzed for glutathione and glucose. Insulin was then administered and all were given breakfast. Eleven of the 42 patients were treated without insulin. A second blood sample was drawn 3 hours later and was analyzed for glutathione. Pre- and post-insulin hematocrit values were determined in Patients 33 to 42 inclusive.

GROUP 3 consisted of 13 patients hospitalized in neuropsychiatric wards. All were physically normal. The diagnosis was schizophrenia in all but 2, and these were severe psychoneurotics. They were divided into two sub-groups depending on whether or not they were receiving insulin therapy.

Group 3A consisted of 4 patients who did not receive insulin. A fasting blood sample was drawn on each one and analyzed for glutathione. Two were schizophrenics and 2 were psychoneurotics.

Group 3B was made up of 9 schizophrenic patients receiving insulin shock therapy. They received increasing doses of crystalline insulin daily from Monday through Friday inclusive over a period of 26 days. Insulin was not given on Saturdays or Sundays. Fasting blood samples were analyzed for glucose and glutathione. Each patient was then given the prescribed amount (between 100 and 580 units) of insulin subcutaneously. A second blood specimen was taken shortly before termination of the hypoglycemia. The period of therapy varied between 3½ and 4 hours. This experiment was repeated 1 to 3 times in the same individual. Pre- and post-insulin hematocrit determinations were made on several occasions.

GROUP 4 consisted of 1 hospitalized patient who had diabetes mellitus with marked insulin resistance and pulmonary tuberculosis. He had received 300 to 520 units of insulin daily for 6 months prior to this period of observation, and during this experiment required 340 to 520 units daily. Glutathione and glucose were determined on fasting blood samples. He was then given his prescribed dose of insulin and breakfast, and a second blood glutathione was determined 3 hours later. This experiment was repeated 4 times, and on one day glutathione was determined 3 hours after each dose of insulin (150 units three times daily). This was a true case of insulin resistance as the patient had been studied intensively in hopes of determining the cause. His pulmonary tuberculosis was well controlled.

Blood glutathione was determined by the method of Woodward and Fry[16]. The Folin Wu method for blood glucose was used[7]. Heparinized blood was used in all instances.

Results. GROUP 1. The fasting blood glutathione values in normal people (Table 1) were found to vary between 26.9 and 41.4 mg. per 100 cc. of whole blood averaging 35.4 mg. per 100 cc. Woodward and Fry state that the normal values by this method vary between 25 and 41 mg. with an average of 34 mg. per 100 cc.

TABLE 1. NORMAL FASTING BLOOD GLUTATHIONE VALUES

Patient	*Glutathione mgms. per 100 cc.*
1. R.C.	31.0
2. B.F.	33.6
3. R.W.	39.2
4. J.S.	36.5
5. P.S.	31.2
6. J.S.	26.9
7. W.D.	35.8
8. B.H.	39.2
9. R.C.	41.4
10. J.H.	40.1
average	35.4

GROUP 2. The blood glutathione values found in the diabetic patients is recorded in Table 2. All were well within the normal range with the exception of one (No. 9) who was found to be about 7 mg. per 100 cc. above the upper limit of normal. There was no significant difference between those who had been receiving insulin and those treated with diet only.

Insulin did not appear to cause any consistent change in the blood glutathione 3 hours after administration. In some instances there was an increase over the fasting value, and in others a diminution. A negative change in the post-insulin value occurred almost as frequently as a positive. Of the 38 patients who received insulin, 15 were positive, 13 negative, and 3 showed no change in the post-insulin value of blood glutathione. The 3-hour glutathione changes were similar in those who did not receive insulin. Of 11 such patients, there were 4 positive and 7 negative changes in the 3-hour specimen.

In Patients 1 to 9, post-insulin blood glucose values were determined in addition to pre-insulin values, and it can be seen that there was no correlation between the height of the blood sugar and the glutathione level. There was no correlation between the hematocrit

TABLE 2.—FASTING BLOOD GLUTATHIONE AND GLUCOSE VALUES OF DIABETIC PATIENTS, AND THE EFFECT OF INSULIN ON THE BLOOD GLUTATHIONE 3 HOURS AFTER ADMINISTRATION

Patient	*Insulin dosage* units*	*Blood Glucose mg./100 cc. insulin* pre	post	*Glutathione mg./100 cc. fasting*	*Glutathione mg./100 cc. post-insulin*	*Glutathione mg./100 cc. change*	*Hematocrit insulin* pre	post
1. S.B.	Glo–30	298	412	34.6	35.8	1.2		
2. H.D.	0	145	110	37.1	38.3	1.2		
3. R.S.	Pzi–20 Reg–40	149	306	37.1	37.1	0		
4. R.C.	Pzi–12	137	209	37.1	38.6	1.5		
5. H.S.	Pzi–15	141	127	45.0	43.8	−1.2		
6. J.A.	0	126	133	39.5	35.5	−4.0		
7. N.R.	Pzi–20	247	275	38.0	37.7	−0.3		
8. T.M.	Pzi–40	110	133	36.8	38.6	1.8		
9. D.E.	0	126	106	48.4	49.0	0.6		
10. W.T.	Glo–50	58		34.4	40.1	5.7		
11. N.R.	Pzi–30	298		38.3	38.3	0		
12. L.S.	Pzi–25	161		36.5	31.9	−4.6		
13. C.D.	Pzi–30	83		38.2	42.0	3.8		
14. S.V.	Pzi–34	106		44.6	42.8	−1.8		
15. E.S.	Pzi–45 Reg–15	129		36.2	39.8	3.6		
16. A.G.	Pzi–20	141		37.2	37.0	−0.2		
17. C.G.	Pzi–40	141		36.8	38.6	1.8		
18. S.W.	Pzi–15 Glo–35	166		39.8	40.2	0.4		
19. J.V.	0	161		34.9	32.5	−2.4		
20. H.R.	0	100		35.0	32.6	−2.4		
21. H.K.	Pzi–35	247		44.1	42.9	−1.2		
22. H.S.	0	161		35.2	37.6	2.4		
23. A.G.	Pzi–65	232		31.0	26.6	−4.4		
24. R.B.	Pzi–20	91		29.5	28.8	−0.7		
25. P.D.	Glo–30	157		36.8	35.5	−1.3		
26. S.B.	Glo–30 Reg–10	412		32.8	32.8	0		
27. R.J.	Pzi–20	180		36.8	33.7	−3.1		
28. G.F.	Reg–20 Pzi–10	149		33.1	30.9	−2.2		
29. J.A.	0	147		37.7	31.9	−5.8		
30. W.S.	0	255		33.1	32.1	−1.0		
31. A.C.	Pzi–15	141		33.7	29.7	−4.0		
32. W.S.	Pzi–5	149		43.8	39.8	−4.0		
33. A.G.	Pzi–65	190		28.5	31.5	3.0	45	46
34. W.T.	Glo–45	196		31.9	32.8	0.9	43	42
35. A.H.	0	182		33.1	31.9	−1.2	43	44
36. R.O.	Glo–30 Pzi–50	112		37.1	43.8	5.7	45	47
37. C.C.	Pzi–40	145		31.9	36.4	4.5	49	49
38. G.J.	Pzi–45	95		27.6	29.1	1.5	44	45
39. D.M.	0	104		38.6	40.4	1.8	50	50
40. R.C.	Pzi–10	137		33.7	35.5	1.8	43	44
41. B.M.	0	133		28.2	27.3	−0.9	45	46
42. L.S.	Pzi–45	196		28.5	31.5	3.0	40	42

* Protamine Zn insulin
Globin insulin ■
Regular insulin

and glutathione changes as shown by Patients 33 to 42 inclusive.

Group 3. The pre- and post-insulin glutathione values in neuropsychiatric patients receiving large doses of insulin are shown in Table 3. There was an increase 3½ to 4 hours after receiving insulin in all cases varying from 0.3 to 6.2 mg. per 100 cc. The average increase in response to doses of crystalline insulin ranging from 100 to 580 units was 3.4 mg. per 100 cc. By comparing the increase in each patient during the 3 experiments it can be seen that the change was not constant for each individual. There was no correlation between the dosage of insulin and the magnitude of the increase of blood glutathione. As an example, Patient 1 received 325 units of insulin and had an increase of 3.0 mg. per 100 cc. In 2 later experiments he had an increase of 4.2 and 6.1 mg. per 100 cc. while receiving 315 and 250 units of insulin respectively.

In all cases in which hematocrit determinations were performed there was an increase of the post-insulin value ranging from 1 to 10, averaging 4.4. This mild hemoconcentration was attributed to the marked perspiration caused by the prolonged hypoglycemia. In view of the fact that glutathione is thought to reside in the red cell only and not in the plasma, it is believed that the post-insulin elevation of blood glutathione (average 3.3 mg. per 100 cc.) was due to the hemoconcentration rather than to a direct insulin effect. There was no correlation between the magnitude of the increase of hematocrit and the increase of glutathione.

Table 4 lists the fasting glutathione values of a group of neuropsychiatric patients who did not receive any insulin therapy. The average was 37.1 mg. per 100 cc. in comparison to an average of 32.9 for the group that received insulin over a 26-day period.

TABLE 3.—FASTING BLOOD GLUTATHIONE LEVELS OF SCHIZOPHRENIC PATIENTS AND THE EFFECT OF CRYSTALLINE INSULIN ON THE LEVEL 3 TO 4 HOURS AFTER ADMINISTRATION

Patient	*Insulin dosage* units*	*Glutathione mg./100 cc. fasting*	*Glutathione mg./100 cc. post-insulin*	*Glutathione mg./100 cc. change*	*Hematocrit insulin* pre	post	*Blood Glucose mg./100 cc. fasting*
Experiment 1							
1. J.K.	325	25.8	28.8	3.0			116
2. G.G.	400	27.9	28.4	0.5			111
3. C.M.	400	28.2	30.7	2.5			119
4. J.M.	285	40.5	45.4	4.9			112
5. G.C.	210	40.2	44.9	4.7			122
6. R.B.	260	36.5					103
7. R.M.	390	26.0	29.4	3.4			122
Experiment 2							
1. J.K.	315	29.4	33.6	4.2	46	49	
2. G.G.	420	25.6	28.8	3.2	44	50	
3. C.M.	430	29.4	30.6	1.2	46	49	
4. J.M.	285	40.4	44.0	3.6	42	52	
5. G.C.	220	39.2	41.6	2.4	39	50	
6. R.B.	300	37.4	41.0	3.6	48	49	
7. F.P.	110	33.6	39.8	6.2	48	49	
8. E.B.	100	32.2	36.8	4.6		49	
Experiment 3							
1. J.K.	250	24.5	30.6	6.1			129
2. G.G.	520	24.5	28.2	3.7			106
3. C.M.	580	26.0	29.4	3.4			103
4. J.M.		40.7					70
5. G.C.	370	41.1	44.9	3.8			116
6. R.B.	500	35.2	39.5	4.3			103
7. F.P.	470	38.1	40.2	2.1			114
8. E.B.	340	36.8	37.1	0.3			116

* Crystalline insulin

TABLE 4.—FASTING BLOOD GLUTATHIONE VALUES IN SCHIZOPHRENIC PATIENTS WHO DID NOT RECEIVE INSULIN THERAPY

Patient	*Glutathione mg. % fasting*
1. B.T.	43.1
2. A.B.	39.3
3. A.P.	30.5
4. H.L.	35.6
average	37.1

GROUP 4. The data pertaining to the diabetic insulin resistant patient are recorded in Table 5. This patient's fasting blood glutathione values were rather constant and within the normal range. In 3 instances he had a post-insulin glutathione rise over the fasting value. This elevation varied between 4.2 and 5.9 mg. per 100 cc. after receiving 100 to 150 units of crystalline insulin. In a 4th experiment there was a decrease. Determinations of blood glutathione 3 hours after each dose of 150 units of crystalline insulin (3 times) throughout a 12-hour period revealed changes of 1.8 and zero mg. per 100 cc.

TABLE 5.—FASTING BLOOD GLUTATHIONE LEVELS IN A PATIENT WITH DIABETES MELLITUS AND INSULIN RESISTANCE AND THE EFFECT OF INSULIN ON THE BLOOD GLUTATHIONE 3 HOURS AFTER ADMINISTRATION

Date	*Insulin dosage units*	*Glutathione mg./100 cc. fasting*	*Glutathione mg./100 cc. post-insulin*	*Glutathione mg./100 cc. change*
12-30-49	150	33.7	38.0	4.3
1-9-49	150	30.6	34.8	4.2
1-11-50	150	—	31.8	—
	150		33.6	1.8
	150		33.6	0
	70*			
1-25-50	100	30.9	36.8	5.9
2-20-50	100	34.0	31.5	−2.5

* Globin insulin

Discussion. The data presented demonstrate that the blood glutathione is not diminished in diabetes mellitus and is well within the normal limits. This is in agreement with the findings of Dogliotti and Meloni[4] and contrary to the findings of others[1,8,9,13,15,17]. Critical scrutiny of the latter papers does not warrant the conclusion of a diminished glutathione in diabetes mellitus and elevation by insulin.

It has been stated that insulin elevates the blood glutathione, and this may account for the normal values in some diabetic patients when in reality it is diminished. This was not borne out, for the blood glutathione values were no higher in those patients receiving insulin as compared to those who were treated with diet alone. Further proof of this is shown when the glutathione values of the neuropsychiatric patients who did not receive insulin are compared with those who were given large amounts over a 26-day period. There was no significant difference, the average of the non-insulin group was 37.1 mg. per 100 cc. while that of the insulin group was 32.9 mg. In fact, the average was lower in the group that received insulin.

There was no progressive increase in the fasting blood glutathione values of the same individuals during the 26-day period of insulin therapy. If the supposition that insulin caused an elevation of blood glutathione to normal in diabetic patients was correct, one would expect the fasting values to become progressively higher as larger amounts of insulin were administered to the same individual. This is further corroboration of the inability of insulin to influence the blood glutathione.

We could not obtain any conclusive evidence that insulin caused even a temporary elevation of the blood glutathione. The administration of insulin to diabetic patients did not produce any consistent changes 3 hours later. There were both positive and negative changes in the latter value, and both were of the same magnitude. The same results were obtained in the diabetic patients who were treated without insulin. The differences between the con-

trol and 3-hour samples in all cases were attributed to the small variations that may occur in the same individual at different times rather than to the inherent error of the method. The latter is much smaller than the differences that were found.

The administration of very large doses of crystalline insulin (100 to 580 units) to the schizophrenic patients produced more consistent changes in the post-insulin blood glutathione. There usually was an increase averaging 3.3 mg. per 100 cc. This was not thought to be significant and was attributed to both a slight increase of the post-insulin hematocrit and the normal variation of the blood glutathione. There was no consistency in the magnitude of the increase of the blood glutathione in the same individual in each of the 3 experiments. This plus the fact that there was no correlation between the dosage of insulin and the degree of elevation of the glutathione speaks against this being a true insulin effect.

The problem of insulin resistance in diabetes mellitus remains an enigma. In some cases the phenomenon has been attributed to insulin neutralizing substances as demonstrated by Lowell[14]. Our insulin resistant patient was found to have insulin neutralizing substances in his blood. We then thought that the resistance to insulin might be due to an abnormally high blood glutathione. This was based on the observation of the *in vitro* inactivation of insulin by glutathione. The disulfide groups of the insulin molecule are reduced to the sulfhydril form resulting in the destruction of the hypoglycemic action of the hormone[6].

Study of the blood glutathione in the insulin resistant patient revealed it to be well within normal limits. Hence, his resistance could not be attributed to the inactivation of insulin by abnormally large amounts of glutathione. The *in vivo* inactivation of insulin by glutathione has never been demonstrated.

As in the other patients, insulin did not cause any significant change in the blood glutathione. There was no correlation between the dosage of insulin and the fasting blood glutathione. The value remained rather constant. In fact, it was slightly higher while receiving 340 units than on several occasions when he had 520 units of insulin daily. This is further substantiation of the fact that insulin therapy does not elevate the blood glutathione of diabetic patients to spurious normal levels.

This work does not support the concept of a diminished quantity of glutathione being responsible for the destruction or impaired function of the beta cells of the islets of Langerhans in diabetes mellitus. This does not disprove the concept, for low intracellular glutathione levels may not be reflected in the blood level. However, the *in vitro* inactivation of insulin by glutathione argues against the hypothesis. It would be difficult to conceive of diabetes (insulin insufficiency) as being due to a diminished amount of glutathione in the same cell that makes insulin when the former has been shown to irreversibly destroy insulin.

Insulin does not have any effect on the blood glutathione in contradistinction to ACTH which is reported to produce a lowering. These 2 hormones are considered to be antagonistic to each other, but in the realm of glutathione no such antagonism appears to exist.

Summary. Fasting blood glutathione values were determined in diabetic patients treated with insulin and by diet alone. The values were found to be within the limits of normal; 25 to 41 mg. per 100 cc. of whole blood. Insulin was not found to elevate the blood glutathione and cause spurious normal values in diabetes mellitus. There was no correlation between the

blood sugar and the blood glutathione.

Large doses of crystalline insulin varying between 100 and 580 units were not found to have any significant effect upon the blood glutathione of physically normal schizophrenic patients 3 to 4 hours after administration. There was no correlation between the dosage of insulin and the blood glutathione.

The blood glutathione was found to be normal in an insulin resistant patient with diabetes mellitus and pulmonary tuberculosis. It was concluded that his insulin resistance was not due to inactivation of insulin by an abnormally elevated blood glutathione. As in the other patients, insulin did not have any influence on the blood glutathione.

It was concluded that this work does not support the hypothesis of a diminished glutathione being responsible for the destruction or impaired function of the beta cells of the islets of Langerhans in diabetes mellitus.

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SYSTEMIC LUPUS ERYTHEMATOSUS

Some Cutaneous Manifestations Related To The Diffuse Collagenous Diseases

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Lupus erythematosus of the acute and subacute types is a systemic disease in which the skin may or may not be involved[6,14,17]. Its general pathology has related it to a group of conditions among which are Libman-Sacks Syndrome[12,14], scleroderma[11], periarteritis nodosa[2], dermatomyositis[10], rheumatoid arthritis[20], and thromboangiitis obliterans[20]. To the above list, on the basis of allergic and pathologic evidence, Rich[15] has added rheumatic fever, Schonlein-Henoch's purpura, serum sickness and certain drug reactions (notably some aspects of sulfonamide and iodine sensitivity).

The basic pathologic process in all these conditions has been variously described as diffuse collagen disease[11], a primary vasculitis[20], or vascular hyper-sensitivity[15]. In lupus erythematosus many of the clinical and pathologic features of the other members of this group are in evidence from time to time.

It is the purpose of this paper to describe 3 patients in whom a variety of significant skin lesions were noted in the course of what had originally appeared to be lupus erythematosus. These lesions demonstrated the wide variation of tissue reaction to many substances, which is a characteristic feature of disseminate lupus erythematosus and other diseases of this group.

Case Reports. Case 1. D. G., a white woman, aged 25, was seen on August 2, 1938, suffering from an eruption of the face of 4 years' duration and remittent fever for 4 months.

Examination revealed sharply demarcated erythematous, scaling patches on the cheeks, the bridge of the nose, the ears and the scalp. Other findings included cervical adenopathy and enlarged "infected" tonsils.

During hospitalization the patient ran a consistent low-grade afternoon fever (99.6 to 100°F.) The sedimentation rate was 71 mm. per hour (normal reading 5 to 20 mm. per hour), and the leukocyte count was 3500 to 6000. A chest roentgenogram showed a healed primary tuberculous complex. The tuberculin test (1:1,000,000) was positive. Gynecologic examination revealed chronic urethritis, endocervicitis and salpingo-oophoritis. *Staph. aureus hemolyticus*, *Strep. hemolyticus*, *Strep. viridans*, and a non-hemolytic streptococcus were repeatedly cultured from the cervix, the urine, the sinuses and the throat.

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Intradermal testing with extremely dilute concentrations of all these organisms showed pronounced hypersensitivity. The skin biopsy was reported as lupus erythematosus. Several cutaneous exacerbations with fever followed a tonsillectomy, the extraction of an infected tooth, the appearance of an active urinary infection and injections of autogenous vaccines.

After a short remission, new skin lesions began to appear in 1939, later assuming a new character (so-called lupus erythematosus profundus) with deep seated diffuse edematous and inflammatory lesions. Lesions clinically compatible with a diagnosis of papulo-necrotic tuberculid were observed on the extremities. There were joint pains, low-grade fever, leukopenia, pelvic infection, cystitis and left-sided pyelitis. Sulfanilamide was administered, but was discontinued when ulceration of the anus, throat and vagina developed. Dilatation of the left ureter and estrogen therapy precipitated an exacerbation of the skin lesions with fever. In June, 1942, after a remission of 2 years, there was a dissemination of the eruption. A left tubo-ovarian abscess was found for which panhysterectomy was performed. Marked improvement followed this procedure (see report of this case by Rose and Pillsbury[17]). In May, 1945, painful erythema nodosum-like lesions appeared on both lower extremities with intermittent fever. In January, 1947, the patient developed erythematous, purple, tender nodules near both ankles and on the anterior surface of both lower legs. Biopsy of one of these lesions was reported periarteritis nodosa (Dr. Herman Beerman). In September, 1947, marked persistent swelling of both legs, more pronounced on the right, was noted. After a number of weeks it disappeared following 2 injections of tetraethylammonium chloride. In October, 1948, roentgenograms of the lumbar vertebrae, taken because of severe backache, revealed a destructive process, probably tuberculous, involving T-11, T-12, L-3 and L-4. The cutaneous lesions were then entirely quiescent, atrophic, cicatricial and pigmented.

Comment. This case illustrates the clinical succession of several types of lupus erythematosus lesions in the skin, beginning with the chronic discoid variety, punctuated by several bouts of subacute and acute disseminated lupus erythematosus, and marked on one occasion by the development of the profundus variety of this disease[9]. Of particular interest is the development of skin lesions similar to those found at times in other members of the group of systemic diseases mentioned previously. These included papulo-necrotic tuberculid-like lesions[13], erythema nodosum, acute diffuse non-inflammatory swelling of the extremities, and lesions which clinically and histologically were compatible with periarteritis nodosa.

The multiple chronic foci of infection, the acute exacerbations following their disturbances and the varying vascular cutaneous manifestations are features that conform to a general anaphylactoid and vascular pattern.

Case 2. R. L., a white woman, aged 38, was seen on August 10, 1948. She had suffered from an eruption of the face, ears and the V of the neck since 1938, diagnosed as chronic discoid lupus erythematosus. In January, 1948, successive crops of nodules developed, usually associated with chills and fever, and lasting about 4 to 6 weeks. Since the age of 16, she had had attacks of numbness and coldness and blanching of the fingers. Two attacks of afebrile "scarlet fever", the 2nd after the use of a sulfonamide preparation, were diagnosed in 1938 and 1942. With the subsequent administration of a sulfonamide drug 1 year later, a diffuse rash again appeared.

The patient was undernourished, pale and looked older than her stated age. An eruption consisting of white, depressed, scaling scars, was present on the nose, cheeks, left temple, V of the neck, above the right eye and behind the left ear. The legs and, to a lesser extent, the arms and the lower abdomen were the sites of 12 to 15 lesions of a different type in various stages of evolution. The earliest of these were tender, subcutaneous infiltrations 1 to 3 cm. in diameter. Others were attached to and produced redness of the overlying skin. The older lesions were hard, sharply demarcated, non-tender, pea-sized to almond-sized nodules.

The blood picture, except for a hemoglobin of 60%, was normal. The urinalyses were essentially normal. The sedimentation rate was 91 to 100 mm. per hour. Roentgen ray examination of the chest revealed pleural thickening of the left apex and left hilum. An intravenous urogram showed intrarenal calcifications of the right kidney and evidences of an old pyelo-nephritis in the left kidney. A skin biopsy of one of the nodules on the thigh was diagnosed as erythema nodosum. The intra-cutaneous tuberculin

test using 0.005 mg. PPD was positive. The dermatologic consultant's diagnosis was chronic discoid lupus erythematosus, but he felt that the total clinical picture probably represented a change toward the acute disseminate type. There was an occlusion of the left ulnar artery. The cold chamber tests revealed cyclic vasospasms of the Raynaud's type. Two fluffy exudates were found in the fundus of the left eye consistent with the diagnosis of acute disseminate lupus erythematosus. During the patient's hospitalization she ran an intermittent temperature up to 103.2°F. This gradually fell to normal after one month.

In October, 1948, recurrent bouts of fever and new crops of lesions appeared. The patient was presented before the Philadelphia Dermatological Society in November, 1948. It was the opinion of several members of the Society that the nodular eruption represented either a form of periarteritis nodosa or erythema nodosum associated with lupus erythematosus.

Comment. This patient demonstrated the following interesting features: Raynaud's syndrome since the age of 16, evidence of an old kidney and lung infection, and 2 afebrile episodes diagnosed as scarlet fever. The initial episode began at about the time the lesions of lupus erythematosus first appeared on the face, while the 2nd followed the administration of a sulfonamide. Since the patient later developed a similar rash attributed to the administration of a sulfonamide, there is considerable doubt regarding these diagnoses of scarlet fever. The 2nd and 3rd eruptions probably were sulfonamide reactions. The reactivity to the sulfonamides, the vaso-spastic background, the subsequent development of vascular lesions in the form of erythema nodosum, the obliteration of the ulnar artery, and the lesions in the eye-grounds tend to emphasize the basic vascular nature of this process.

Case 3. R. E. Z., a white woman, aged 27, was admitted to St. Joseph's Hospital, Reading, Pa., on August 13, 1949. On July 4, 1948, she sustained an excessive "sunburn" of the face, the V of the neck and the extensor surfaces of the forearms following a day's outing. She complained of loss of appetite, low-grade fever, malaise, bouts of mild abdominal cramps, diarrhea and weight loss.

The patient was well-nourished but in great distress. The forehead, cheeks, chin and the presternal and interscapular regions presented an erythematous, papulo-squamous eruption with some tendency toward confluency. A few fine erythematous and purpuric spots were observed on the dorsa of both hands and on the fingertips. The palms exhibited large erythematous and ecchymotic patches. Throughout the first 100 days of hospitalization the patient ran a fluctuating temperature of 100 to 102°F. Thirty days after admission an exacerbation developed with the appearance of areas of cutaneous gangrene involving the digits, arms, legs, face and ear lobes. The lesions on the terminal phalanges ended in gangrene and mummification. Daily blood pressure readings rose gradually from 110/70 to 170/110 just before death. Examinations of the fundi revealed a grade 2 hypertensive retinopathy. About the 75th hospital day the patient developed severe abdominal pains. She died 143 days after admission. The laboratory studies were as follows: Hemoglobin 60 to 69%, leukocytes 3,100 to 11,500, averaging 5000, red blood cells 3.24 million. The differential counts were essentially normal. The platelet counts varied from 169,000 to 283,000. The sedimentation rates were 102 to 158 mm. per hour. Numerous urinalyses revealed a variable specific gravity. Albumin was 1 to 4 +. There were occasional red blood cells, white blood cells and casts in the urine. The non-protein nitrogen on the 23rd day of hospitalization was 25 mg. per 100 cc. of blood. After 140 days it was 90 mg. Skin biopsy of a papule on the left deltoid region was diagnosed as consistent with acute disseminate lupus erythematosus.

Autopsy findings: plastic pericarditis, vegetative endocarditis involving the mitral valve, chronic fibrous pleurisy of the right pleura, generalized acute peritonitis, perforated posterior pre-pyloric gastric ulcer, 2 small splenic infarcts, passive congestion of the spleen, liver, kidneys and lungs. The pertinent histologic findings were renal infarction associated with wire loop glomerular structures (disseminated lupus), degeneration of the adrenals and chronic ulceration of the toes (gangrene). The cause of death was generalized peritonitis secondary to perforated gastric ulcer associated with disseminated lupus.

Comment. This patient suffered from the acute fulminating type of disseminate lupus erythematosus with vascular complications of hypertension, retinopathy, nephritis, adhesive pericarditis,

valvular endocarditis and widespread purpura. Similar types of vascular complications are also common manifestations in periarteritis nodosa. The marked degeneration of the adrenals found here is of interest in relating this disease to Selye's experimental "general adaptation syndrome"[18] where advanced adrenal changes have been described in association with hypertension and diffuse vascular damage.

Discussion. Some cutaneous manifestations of a rather unusual character in systemic lupus erythematosus are recorded herein in order to emphasize the connection between this disease and other generalized vascular phenomena, such as periarteritis nodosa, dermatomyositis, scleroderma, thromboangiitis obliterans, rheumatoid arthritis, anaphylactoid purpura and vascular reactivity to sulfonamides. In Cases 1 and 2 the sulfonamides may have activated a focus of infection to produce the acute manifestations of lupus erythematosus. Rich's[15] experimental evidence emphasizes the role of the sulfonamides in precipitating lesions of this type in an appropriately prepared tissue. Exacerbations of the chronic discoid type of lupus erythematosus following sulfonamide therapy have been described by Barber[3], and an eruption simulating lupus erythematosus has been reported by Hoffman[7] as a sensitization reaction to sulfathiazole. The multiple foci of infection and a high degree of reactivity to disturbance of these, or to the injection of bacteria derived from these foci, indicates a widespread hyperergic state in accordance with Rich's[15] concept of generalized vascular allergy. The idea that multiple antigen reactivity plays a role in precipitating lupus erythematosus was emphasized by Ayavazian and Badger[1] who reported a relatively high incidence of acute lupus erythematosus among a group of student nurses following reactions to a series of routine prophylactic procedures such as smallpox vaccination and diphtheria and scarlet fever immunizations. Experimental evidence for an unusual degree of antibody response in lupus erythematosus following blood transfusion has been offered by Callender and Race[4]. In addition, Hopkins and Burky[8] have described an enhanced reactivity of the skin of patients with lupus erythematosus to staphylococcus toxin.

The presence of Raynaud's syndrome tends to link lupus erythematosus with acrosclerosis. Obliteration of the ulnar artery may show its association with Buerger's disease. In this connection, the report of Hausner and Allen[5] called attention to the widespread nature of some cases of thromboangiitis obliterans. Case 3 emphasizes the similarity between acute lupus erythematosus and periarteritis nodosa in the form of hypertension, vascular retinopathy, nephritis and purpura.

These 3 cases seem to supply evidence for the concept that the systemic varieties of lupus erythematosus represent a generalized vascular and allergic type of reactivity in which multiple allergens may play an important role. The disease may be induced by the action of numerous foci of infection supplying a susceptible vascular tree with a continuous bombardment of allergenic material. Certain influences, such as exposure to sunlight or the use of sulfonamides, may act as precipitating factors on a well prepared or a specifically charged terrain[19]. Since lupus erythematosus is theoretically related to the other diffuse vascular anaphylactoid disturbances, it is not strange that an occasional cutaneous manifestation of this disease mimics those found in some other members of this group.

The role that the adrenals may play in the etiology of lupus erythematosus, as well as of other members of this group, has been suggested by the work of Selye[18] on the "adaptation syndrome", where experimentally diffuse

vascular changes have been observed. In addition, the recent report of Hench *et al.*[6] on the effect of cortisone and pituitary adreno-corticotropic hormone (ACTH) on rheumatoid arthritis has led to a suggestion of the possible action of these agents on lupus erythematosus. Already preliminary work has indicated that these substances may be of value in inducing remissions in acute erythematosus.

Summary and Conclusions. 1. Three cases of systemic lupus erythematosus are described with some unusual cutaneous manifestations having a common pathologic denominator in vascular damage. These lesions included a papulo-necrotic tuberculid-like eruption, erythema nodosum, periarteritis nodosa, gross vascular occlusion, Raynaud's syndrome, diffuse swelling of the legs and gross gangrene of the skin. They are presented here as cutaneous examples of the connection between this disease and a group of related entities in which diffuse collagenous and vascular changes are the fundamental pathologic features.

2. Clinical evidence is presented to re-emphasize the role that widespread hyperergic reactivity to multitudinous antigens plays in inducing these vascular alterations.

3. Certain precipitating factors such as sunlight and the sulfonamides are suggested as possible inciting elements in the exacerbations of this disease, presumably acting in an appropriately prepared vascular terrain.

4. The important role of the adrenal cortex in vascular physiology, as elucidated by Selye's "alarm reaction"[18] studies, and the effect of cortisone in inducing remissions in acute lupus erythematosus are collateral suggestive evidence that the vascular system is the basic pathologic locus of this disease.

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CHRONIC GLOMERULONEPHRITIS WITH SEVERE RENAL TUBULAR CALCIFICATION *

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An instance of marked renal calcification associated with chronic glomerulonephritis was recently observed by us, and a search of the literature indicated that this is a finding of extreme rarity. In fact, only one previous case has been reported[2]. The calcification in our case, as in the previously reported one, was limited to the convoluted tubules and was of such an extreme degree as to be clearly visible in plain roentgenograms of the abdomen (Fig. 1). Vaughan *et al.* reviewed the subject, including the biochemical considerations, very thoroughly and it is not deemed necessary to discuss these aspects in detail. However, the unusual nature of the disease warrants recording in the literature.

Case Report. The patient, a 30-year old white male, entered the Veterans Administration Hospital on June 8, 1949, with the complaint of acute abdominal pain which had been present for 48 hours. Prior to this he had been working daily and had noted no abnormalities except for some increase in fatigability. The pain was in the mid epigastrium and had awakened him from sleep forty-eight hours before admission. It persisted the following day and was of similar intensity, and on that day no additional symptoms were noted. Twenty-four hours prior to admission the patient had retired for the night with the above symptoms, but on awakening the following morning the abdominal pain had disappeared and he had felt well enough to return to work. Shortly after returning to work, the midepigastric pain recurred and was severe enough to cause the patient to seek aid from his physician, who immediately hospitalized him at another hospital. Later in the day the patient was referred to this institution.

Past history revealed that the patient had enjoyed excellent health until 1940, at which time he had an episode of illness, characterized by edema of the abdomen, scrotum, and lower extremities, associated with "coffee colored" urine. He was confined to bed at that time for 2 months and apparently made an excellent recovery, for he returned to work, and was accepted into the armed forces in 1941. Approximately 1 year following his entry into the army, he became ill again with symptoms of generalized abdominal pain, swelling of the lower extremities and dark urine. The patient was hospitalized for 10 months, during which time he was told that his blood pressure was elevated. He was subsequently discharged from the service with the diagnoses of chronic glomerulonephritis and malaria. He then returned to his job as a telephone lineman

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and remained in good health until this present episode. The body weight had been constant.

Physical examination on admission revealed a dehydrated, very pale white male about 30 years of age. Vomiting occurred several times during the examination. His face appeared slightly puffy, especially about the eyes. The blood pressure was 130/80, the pulse 100 and the temperature 102. He had generalized abdominal tenderness, which was more marked in the upper left quadrant. Considerable muscle splinting of the abdomen was noted, but no distention was present. Bowel sounds were heard, and rectal examination was negative.

Laboratory studies on admission revealed the following: The urine had a specific gravity of 1.010 and contained many granular casts, 2 plus albumin, 20 pus cells per high power field and numerous red blood cells per high power field. The red blood count was found to be 2,540,000/c.mm. and the hemoglobin 8.8 gm. The leukocytes numbered 6,200/c.mm. with a normal differential count. The nonprotein nitrogen was 150 mg./100 cc., the serum calcium 9 mg./100 cc., the CO_2 combining power 27 volumes %, the serum phosphorus 4.1 mg., the blood chloride 248 mg., and the blood sodium chloride 409 mg. The total serum protein was 5.73 gm. with 3.0 gm. of albumin and 2.73 gm. of globulin. A 24-hour urine specimen contained 9 mg. of calcium/100 cc. A plain film of the abdomen was taken, and it was noted that the kidney shadows were very clearly outlined because of diffuse homogeneous calcification (Fig. 1). The kidney shadows were unusually small, the right measuring 10 by 5 cm. and the left 11 by 4 cm.

Because of the acidosis, anemia, and persistent vomiting, the patient was treated with intravenous feedings. These consisted of daily blood transfusions (250 cc.), lactate Ringer's solution, 1/6 molar lactate, small amounts of normal saline and 10% glucose. The patient was given a total of 2500 cc. daily in varying amounts of the above fluids according to the blood chemistry and calculated loss of fluid. During the first week of the hospital stay the total urinary output varied from 1900 cc. to 4450 cc. every 24 hours, and thereafter the output was approximately 2000 cc. per day. Following the first week the patient was able to take oral fluids and the abdominal symptoms cleared. The temperature returned to normal on the 8th hospital day. The intravenous feedings were discontinued at that time.

Another urine analysis revealed persistent

FIG. 1.—Roentgenogram of abdomen, showing marked calcification of kidneys and reduction in their size.

albuminuria, white blood cells and 8 to 10 red blood cells per high power field. The specific gravity remained fixed at 1.010. The nonprotein nitrogen fell to 100 mg. and remained at that level until discharge. The CO_2 combining power rose to 50 volumes %. The blood sodium chloride rose to 550 mg. and the blood chloride to 336 mg. The anemia persisted in spite of 1500 cc. of whole blood which was given shortly before discharge. The urea clearance was found to be 16% of normal and the phenolsulphonephthalein test revealed that 5% of the dye was excreted in one-half hour.

The patient was released at his own request on the 28th hospital day. Fifteen days later he returned to the hospital in marked acidosis with severe suppression of urine. On this admission the urine analysis disclosed a specific gravity of 1.012; 4 plus albumin; 6 to 8 white blood cells per high power field; and 25 to 50 red blood cells per high power field. The erythrocyte count was 3,500,000/c.mm. and the leukocyte count 16,000/c.mm. with a normal differential. The nonprotein nitrogen was 375 mg. and the CO_2 combining power was 26 volumes %. The blood calcium was 8 mg. and the inorganic phosphorus was 3.85 mg. The patient was critically ill and vomited frequently. The urinary output was recorded as being none on the first two hospital days and approximately 1300 cc. on the 3rd hospital day. On the 4th hospital day the temperature rose to 103, and the patient died.

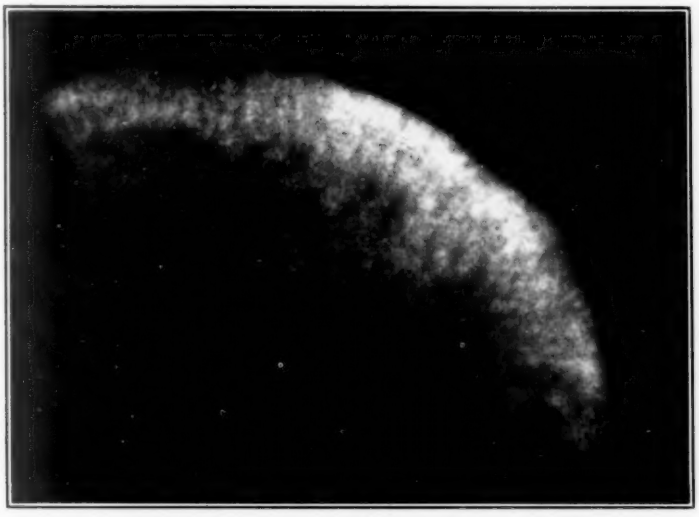

FIG. 2.—Roentgenogram of a portion of a kidney, showing calcification of cortex.

At NECROPSY both kidneys were found to be diminished in size, each weighing 90 gm. The surfaces were roughened and granular in appearance. The capsules stripped with little difficulty. When the capsules were stripped, it was noted that there were many yellow, tiny, granular projections each less than 1 mm. in diameter on the surfaces. The consistency of the kidneys was firm. On cut section both the cortices and medullae were narrowed. Throughout the renal cortex there were numerous deposits of yellowish gritty material, which tended to be arranged in linear strands. It was not present in the medullary pyramids, although it was seen and felt in the cortical substance which dipped downward between adjacent pyramids. These findings are clearly shown in a roentgenogram of a portion of one of the kidneys (Fig. 2). This was a generalized change but appeared to be somewhat more marked at the lower poles of the kidneys than at the upper poles. The architectural details were obscured, and there appeared to be an increase in the amount of fibrous tissue. The pelves and ureters were essentially normal. There was no evidence of disease of the lower genito-urinary tract. The heart weighed 440 gm., and there was a slight increase in the thickness of the left ventricular myocardium. No other changes were noted in the heart. The parathyroid glands were unfortunately not removed.

MICROSCOPICALLY, the kidneys were found to be the seat of extensive disease. Almost all of the glomeruli were abnormal in appearance. Some of them showed little evidence of

chronic disease, but they were ischemic and often contained an increased number of polymorphonuclear neutrophilic leukocytes. Most of the glomeruli, however, showed evidence of long-standing change. Fibrosis and thickening of the capsules, often in crescentic form, was seen commonly (Fig. 3). Some of the crescents seen in the Bowman's capsules were in the nature of epithelial proliferation (Fig. 4). Many of the glomeruli

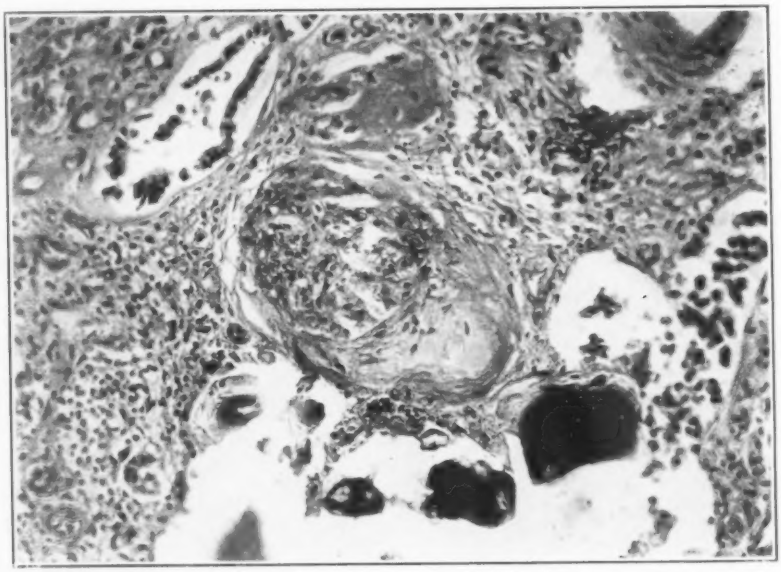

FIG. 3.—Photomicrograph of glomerulus, showing markedly fibrotic crescent. Calcification in adjacent tubules is clearly visible. (× 600)

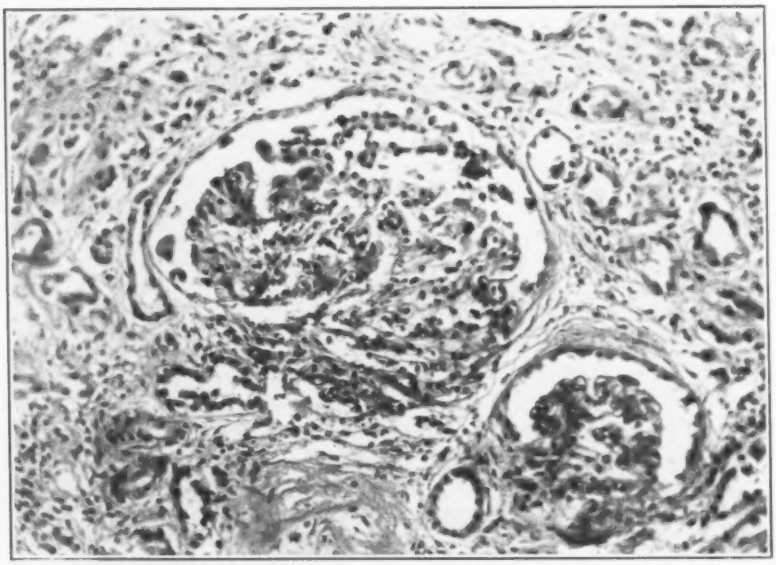

FIG. 4.—Photomicrograph of glomerulus, showing epithelial proliferation in Bowman's capsule. There is some artefactual shrinkage. (× 600)

were fibrotic and partially hyalinized. Numerous adhesions were present between the glomerular tufts and the capsular epithelium. The tubules showed very extensive changes. Some of them exhibited degenerative changes with swelling of the cytoplasm and loss of nuclei. Many were dilated. The most striking finding was very extensive calcification of the renal tubules (Fig. 5). This calcification was in the form of large amorphous masses and appeared to be chiefly in the distal convoluted tubules. In many of them the calcification was so marked as to completely fill the tubular lumens. In those tubules where the calcification was most marked, the epithelium was largely degenerated. A considerable degree of interstitial fibrosis was present and lymphocytes were seen in moderate numbers within the connective tissue. The arterioles were thickened and frequently hyalinized and their lumens were narrowed. This was particularly seen in the preglomerular arterioles. The pathologic diagnoses were chronic glomerulonephritis with marked calcification of renal tubules and hypertrophy of left ventricle.

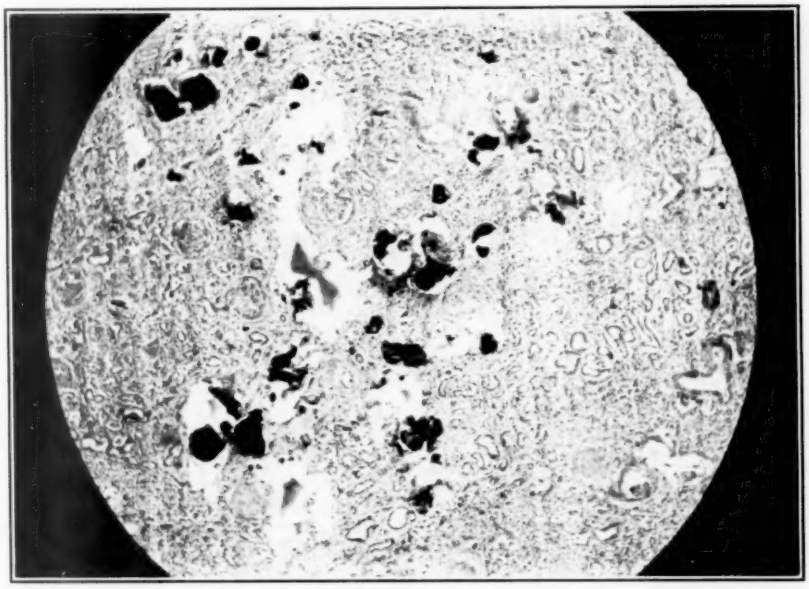

FIG. 5.—Photomicrograph of section of kidney, showing extreme degree of tubular calcification. All of the dark masses are calcific material in the tubules. (× 160)

Discussion. The extreme degree of tubular disease noted in this patient is very unusual, in fact almost unique, in chronic glomerulonephritis. No mention of such a lesion is found in the standard text books on renal disease. The extreme rarity of this lesion brings up the possibility that the tubular degeneration and calcification are unrelated to the glomerulonephritis. We have had the opportunity of studying an instance of acute lower nephron nephrosis following administration of a sulfonamide in which there was very severe tubular necrosis with marked calcium deposition. No significant glomerular lesions were noted. Hoffman[1] has observed another instance of lower nephron nephrosis in which calcification of both kidneys was noted on Roentgen-ray films taken several months after subsidence of the acute renal insufficiency. Although in the present case there was no history of lower nephron nephrosis, it is possible that there had been such an episode and that the calcification of the renal tubules was not an integral part of the chronic glomerulonephritis. In the instance reported by Vaughan *et al.* there

was a history of excessive calcium ingestion, but no definite history of such a dietary abnormality could be obtained in our patient. The parathyroid glands were probably not implicated in the pathogenesis of this lesion because of the normal serum and urinary calcium levels, the normal serum phosphorus determinations, and the absence of metastatic calcification.

Summary. 1. An instance of chronic diffuse glomerulonephritis with marked tubular calcification is described.

2. Mention is made of the possible role of lower nephron nephrosis in producing the calcific tubular lesion.

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THE EPIDEMIOLOGY AND SOCIAL SIGNIFICANCE OF ATMOSPHERIC SMOKE POLLUTION

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THE purity of the air we breathe is of great interest to those whose aim is the improvement of the public health. Some think this purity as important to our well being as that of the water we drink and the food we eat. Within industry, scientific investigation has established this contention beyond doubt. In spite of almost equal attention, the air of our towns and cities remains to be as fully evaluated.

This review is concerned with a limited aspect of atmospheric pollution, that which occurs in urban communities and is due to smoke; and only indirectly with the many contaminants other than smoke introduced into air in the course of industrial processes. Pure air has been defined as containing only its chemical components in proper proportion and free from any air-borne foreign substances whatever[53], a state of perfection only approached in mid-ocean and on mountain tops. Pollution to some degree is almost universal, and commonly is due to a large variety of causes. Smoke J. S. Owens[80] has described as "the mixture of flue gases and suspended particles of solid or liquid matter which is produced by certain fuels in the process of combustion." The chief fuels are coal, oil, and gasoline.

Even before the industrial revolution, coal had considerable use for domestic purposes where supplies of wood were inadequate. Coal has been mined in England for at least a thousand years, and throughout that history people have disliked its smoke and

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feared its effect on their health[29, 31, 62]. The production and consumption of coal soared as the mechanical inventions of the eighteenth century became utilized in industry. A rapidly increasing population, made possible by rising productivity, crowded into the cities. More and more coal went into more and more factory furnaces, ships, and locomotives, and into more and more home fires[20b].

It seems likely that a climax in smoke pollution of atmosphere may have been reached in England in the latter half of the nineteenth century, and in America at the turn of the present century. Industrialization and populations have continued to increase, oil and gasoline now contribute their products of combustion, but the sources of pollution have become less concentrated, and gas and electricity have become more popular, especially for domestic purposes. In addition there has been great progress in the scientific control of atmospheric smoke pollution, stimulated by the desire for economy on the part of the consumer, and for the abatement of a nuisance by the population at large.

Broadly speaking, there are three main sources of smoke: domestic, industrial, and from transport. The domestic source is sometimes the only one, frequently the main one, and always important. The precise part of smoke of domestic origin in the total problem is determined by climate and the degree to which coal is used in the home. The contribution of industry depends on the number of factories, foundries, smelters, coal mines, gas works, and electrical power stations. Rail and water transport are frequently important sources of smoke and road transport contributes through fumes from the combustion of oil and gasoline.

Few people really like smoke, though many see it as the inevitable accompaniment of industrial activity, without which there can be no prosperity in an industrial community. For at least a century, investigators have tried to evaluate its alleged detrimental effects on climate, health, animals, vegetation, buildings, and other structures. For the most part investigators have confined themselves to one of these fields. Only rarely have their results been pooled and the total cost of smoke assessed. Very few indeed have appreciated the desirability of evaluating this cost in terms of man's total ecology.

The natural history of every mass disease exhibits constant variations in distribution and frequency in response to continually changing conditions. The epidemic is a relatively bizarre and unusual episode in the course of a disease and quite atypical of the endemic or usual state of affairs. Smog, too, produces its epidemics[77], the most notorious of which occurred in the Meuse Valley in 1930[30] and in Donora, Pennsylvania, in 1948[78]. It seems reasonable to suggest that these catastrophes stand in much the same relationship to smoke pollution as do outbreaks of food poisoning to Salmonella infection. In both situations, complete understanding is almost certainly dependent upon a study of the chronic endemic problems.

Within a few days of onset of exceptionally thick fog, in the Belgian incident 64 people died and in the American, 20, and in both instances hundreds became severely ill. The population at risk was about 13,000 in Donora and unknown in the Meuse Valley, though it was estimated that the deaths represented a tenfold greater than normal mortality in the municipalities concerned. It is probable that there had been at least one other similar episode in the Meuse during the period January 14 to January 20, 1911, and it is also possible that atmospheric

conditions may have contributed to the increased death rate from cardiovascular disease in Donora in April, 1945.

Both valleys have many features in common, particularly in the nature and degree of industrialization and in the relatively greater contributions of industrial, as opposed to domestic, sources to atmospheric pollution. Both epidemics were precipitated by almost identical meteorological conditions.

Largely as a result of the Donora disaster, there exists at this time considerable public and official interest in the problems of atmospheric pollution. It is natural that with this as the stimulus, emphasis is being placed on air pollution as a hazard to health. Dyktor[25] has noted with regret that there is greater interest in the results of air pollution than in its causes. It is the total well being of man that must be considered, and that depends not only upon the state of his lungs, but upon the sources of his livelihood.

Epidemiology, which has been defined as medical ecology[33], is concerned with the study of community health in its broadest sense, in relation to a total environment which has physical, biologic, and social components. It seems probable, therefore, that the epidemiologic method can contribute in an approach to a problem so intimately connected with many aspects of health, wealth, and happiness.

The Epidemiologic Problems. The usual situation in mass pathology of human populations is to have at hand an established disease entity. The epidemiologic problem resolves into a search for the agent of disease that incites the process, or if that be known, into a study of its character and activities. The second objective is an analysis of those other environmental factors which act in determining the incidence and prevalence of the condition. In the present instance, the position is reversed. The start is with a suspected agent to which man, the host, is subjected under a variety of environmental conditions, but with no clear certainty that any disease results. Without demonstrated indices of disease, be they of death, disability, or defect, further exploration is purposeless. The first need is to examine those diseases which seem to have a possible relationship to atmospheric smoke pollution or have been suspected of such an association.

Objection has been made that smoke produces no disease peculiar to itself, and is but a contributing factor in diseases having other causative factors[62]. This is to lose sight of the ecologic concept of mass disease[34], that disease is the resultant of many forces that arise variously from a host and from the environment in which the host lives. Multiple causation of origin and course is a principle, applying to all mass disease and mass injury. The direct agent of disease is but one feature of a disease-producing complex that lies within environment. For convenience it is frequently detached for separate study, principally because that fits so well with clinical investigation based on a specific etiology. Smoke, or any other potential agent of disease, thus expectedly acts in association with many other causative factors, sometimes dominantly and sometimes in a secondary role.

Atmospheric smoke is not one, but a mixture, of many potential agents, and the concentration of each varies in time and place. Each requires definition and study in order that an accurate dosage scale may be determined in terms of concentration multiplied by time of exposure. The rhythm and frequency of dosage may be of importance. Further information is required of the extent to which the agents present are antagonistic or synergistic in their action.

In the study of the host factor, attention is directed to the influence of

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age, sex, and race in determining the power of the agent to harm man. It would be interesting to know something of the occurrence of natural resistance and undue susceptibility, and whether these qualities may be acquired.

The inter-relationship of smoke and environment is complex. Physical factors of weather and terrain greatly influence its concentration. Smoke, in turn, under certain conditions of temperature and humidity produces fog, interferes quantitatively and qualitatively with sunlight, erodes metal and stone, and makes dirty everything with which it comes into contact. Biological factors of the environment do not appear to have any important influence on smoke, but the effect of smoke on animals, plants, and bacteria may be considerable. Socio-economic factors related to degree and nature of industrialization, methods of heating, crowding and population density, and means of transport are measurable and of obvious importance. The evaluation of the socio-economic effect of smoke, whether by waste of fuel or secondary to its action on the physical and biological environment, presents a difficult and complex task.

The effects of atmospheric smoke pollution on man are both direct and indirect. Health may be influenced indirectly as a result of changes made by smoke in man's environment. Thus, interference with sunlight conceivably results in psychologic and metabolic disturbances, in increased risk of airborne infection, and even in lower general resistance to disease.

The direct effect of agent on host might be due in theory to cutaneous contact, ingestion, or inhalation, though only the last seems likely. We should therefore direct our attention to respiratory disease, both inflammatory and neoplastic.

A difficulty presents itself at this point because names of diseases do not always have the same significance to different people at different times and in different places. There are trends in popularity of disease diagnoses dependent upon current medical theory and upon facilities for diagnosis. This is a common epidemiological problem, but of more than usual importance in this study. Bronchitis, pneumonia, and cardiac diseases are closely related entities[68,84,89]. It may be difficult or impossible to determine either their relative importance or which is the primary condition in an individual. But some decision must be made by the doctor when he writes a sickness or death certificate. Custom varies greatly from place to place, and country to country. It is likely that much of the bronchitis of Great Britain would be diagnosed as pneumonia or cardiac failure in the United States, and of course the reverse is true. A second difficulty is that the frequency of diagnosis of cancer of the respiratory tract varies with many factors other than its absolute and true incidence[49,53]. It is sufficient to say here that we must beware of making comparisons which are not justified.

Smoke as an Agent of Disease. A completely combustible fuel consumed efficiently will result in carbon dioxide and water. These are constant products of combustion and fortunately are harmless. The greater concern is with the products of imperfect fuel and of incomplete combustion. Such fuel gives rise to ash and to volatile substances which are either not inflammable or not always burned completely. Combustible impurities such as sulphur may give rise to additional end products, in this case sulphur dioxide. From incomplete combustion, particles of the fuel and of carbon, gaseous hydrocarbons, and carbon monoxide are liberated.

Polluting substances introduced into

air through smoke are in three main categories:

1. PARTICULATE MATTER: solid particles of carbon, coal and mineral ash. In size they vary from 0.01μ to 100μ or more in diameter[45b]. The particles contain silica in proportion to the silica content of the fuel. The average silica content of the suspended matter, as analyzed in 14 American cities for the years 1930-1933, was found to be 12%[43].

2. LIQUID MATTER: arising from the destructive distillation of the fuel, chemically complex, but presenting as a carbonaceous tarry substance.

3. VAPORS AND GASES: which include gaseous hydrocarbons, carbon monoxide and dioxide, sulphur dioxide and trioxide, oxides of nitrogen, hydrofluoric acid, and fluorides.

Much of this added material eventually settles or is carried to the earth by water. It may be divided into water soluble and insoluble portions. The latter contains most of the particulate and tarry matter, some of which is soluble in carbon bisulphide and then described as "tar". In solution are found sulphur in the form of sulphites and sulphates, chlorine, ammonia, lime, and free acid[20a].

The oxides of sulphur are water-soluble and readily form sulphurous and sulphuric acids in moist air. In the presence of sunlight, sulphurous acid undergoes oxidation to sulphuric acid[45b]. The rate of this change is slow under normal conditions, but is increased when air movement is slight[45a] and by the presence of dust particles which act as a catalyst, particularly when metallic in nature[46b].

Even in cities where pollution is considered severe, the concentration of sulphur dioxide seldom rises above 2 p.p.m.[61]. It has been calculated that in central St. Louis man is subjected to a concentration above 2 p.p.m. for less than 20 days in 60 years[90]. In rare and exceptional circumstances, as in the Meuse Valley in December, 1930, and in Donora in October, 1948, the level may possibly have risen much higher, and, what is probably more important, remained at a high level for several days.

Although much work has been done on air pollution by fluorine and its compounds in certain specific industrial circumstances[1,24,67,71a,71b], little is known of their role in smoke pollution. Crossley[19], in an extensive investigation of the coal of Great Britain, found that its fluorine content varied from 0 to 175 p.p.m. In most cases it was less than 80 p.p.m. During combustion this fluorine is liberated either as hydrofluoric acid or as silicon tetrafluoride. In the destructive distillation of coal in the manufacture of coal gas, the liquor is found to contain most of the fluorine.

The final results of a search for known or presumed carcinogenic substances in the air of 9 British cities are not yet available. Benzpyrene has been detected in all samples so far analyzed[11]. It occurs in concentrations of 0.02μg. to 0.5μg. per cubic meter of air, but it is difficult to say how representative the samples have been, and even more difficult to assess whether the finding has any real significance.

It is not proposed to examine in detail the methods used for the measurement of atmospheric smoke pollution. An excellent description of some of the methods available and of their limitations is given by Silverman[82] and by the Department of Scientific and Industrial Research of Great Britain[20a]. Rough measures of solid deposit and of sulphur dioxide concentration can be obtained fairly readily from the instruments in common use. Accurate methods of measuring the concentration of sulphur dioxide and the number, size, and composition of suspended particles

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per unit volume of air are available, but are not frequently used. All methods, fine and coarse, have their limits of accuracy and of comparability, but most difficult is the problem of the interpretation of their results. The analogy is given by one writer of the thermometer hanging from a tree in the garden. The owner may read it accurately but find that he cannot say to what the temperature refers.

Environmental Determinants of Smoke. The concentration of each of the constituents of smoke present in the atmosphere depends on the balance which exists between rates of production and rates of dissipation. The type of smoke produced is related directly to the nature of the fuel and to the efficiency with which it is burned. This efficiency depends largely on the type of equipment available.

The nature of the fuel or fuels used in any community depends on all those environmental forces, physical, biological and socio-economic, which determine the dominant pattern of life in that area. Little is to be gained here from going too deeply into this ecological maze. Sufficient be it to say that the quantity and nature of the fuels used reflect the degree and type of industrialization, the raw material resources, prosperity, geography, topography, and perhaps most important of all, the climate. Much could be deduced about any city from its smoke records.

Differences might be expected between the pollution problems of cities predominantly oil-burning and those predominantly coal-burning. The principal fuels in Los Angeles are natural gas and oil, and the chief complaint referable to smoke is of conjunctival irritation, a relatively insignificant symptom in coal-burning towns. This correlation between type of fuel and the nature of atmospheric pollution was demonstrated in New York in 1934[69]. A complete record of all fuel movements was kept, and the geographical sources of coal and the division of fuel oil between industrial and domestic consumers were determined.

The sulphur content of coal manifests itself in the atmosphere as sulphur dioxide. The lower the percentage of carbon in coal, the higher is that of ash and of the volatile hydrocarbons which go to build up the particulate and tarry components of pollution, especially when inefficiently burned. Domestic open fires are almost always inefficient and in countries where these are common, bituminous coal is the usual fuel. Industrial installations frequently burn better type fuels such as oil, anthracite, hard coal and coke, and what is more important, more often possess better equipment. Coke differs from the coal from which it is made in having lost almost all the tar and other volatile impurities and thereby retaining a relatively higher proportion of ash. The proportions of nitrogen and sulphur are about the same as in the coal. There is an increased likelihood of incomplete combustion with production of carbon monoxide[20b].

An interesting example of environmental influence on the degree of smoke pollution was seen in Dublin during the last war. An acute coal shortage occurred in 1941 and the level of atmospheric pollution fell to about half that of the 3 preceding years[54a]. Some suggestive evidence of the relatively insignificant contribution which the steel mills make to the dust content of Pittsburgh air was obtained during a steel strike. Using a tape recorder, measurements of dust deposit per hour were found to be almost identical with the mills closed down, 60% working, and fully working[27]. It seems possible that other environmental changes brought about by the strike could have played some part in producing this result.

Weather and climate, which have a

considerable influence on the rate of production of smoke from domestic sources, in conjunction with topographical factors control entirely its rate of dispersal. This knowledge is perhaps the most definite in the entire field of atmospheric pollution; for it has been established by surveys in the Meuse Valley[30], in Leicester[20a], in Donora[78], in Los Angeles[83a,83b], in Pittsburgh[27], and in many other American cities[43,61].

The removal of pollution depends on 2 processes: air movement and precipitation. The interrelation of influences of gravity and air movement on suspended matter is complex. For a technical description of this problem the reader is referred to the work of Baron and others[6], Thomas and others[86], and Church[14].

Air movement may be resolved into vertical and horizontal components. Vertical movement occurs when the surface of the earth and the immediately adjacent air are warmer than the air above. The cold heavy air sinks and the warm air rises. Circulation takes place in much the same way as in a domestic hot water system and is known as turbulence. The horizontal component is produced by any wind that blows and is controlled by physical factors less local in nature. Within limits, the horizontal component is stronger at higher altitudes. Topographical factors greatly influence horizontal air movement. Cities beside the sea or other extensive water surface are seldom without fresh breezes. High pressure areas, especially in valleys and sheltered places, more frequently experience periods when the air is still.

Turbulence is vitally important in the dispersal of smoke. It carries it up from the earth to where winds carry it away. Even in the absence of any substantial horizontal air movement, turbulence introduces a great diluting factor. High smoke concentrations arise only when for some reason these natural processes of dilution are inactive. Vertical movement ceases when the temperature gradient is less than the critical level of at least 1°C cooler per 100 meters' elevation[93]. Below the critical level a state of temperature inversion is said to exist.

The temperature gradient varies with the time of the day and the season of the year and is dependent upon the amount of heat reaching the earth from the sun. During the day, the earth warms up and during the night it cools off. The temperature gradient and turbulence are therefore greatest during the early hours of the afternoon and least in the early hours of the morning.

Smoke concentration is complicated by the fact that it is produced for the most part during the day, and in the words of the Leicester survey[20a], "while the daily cycle of smoke has been for some time attributed to a diarchy of solar phenomena and human habits, the problem of distinguishing their respective spheres of influence has proved difficult." Taking both factors into consideration, it is found that concentration and rates of deposit are highest in the morning and in the late afternoon.

In the winter months, the sun shines for fewer hours and its rays pass through the atmosphere so obliquely that less heat reaches the earth. Diurnal temperature change is therefore least during the winter and air stasis occurs most frequently in November and December when the weather is cold, but before the winter gales.

Thus, at a time when most smoke is being produced, meteorological conditions are most favorable to air stasis and to the building up of high smoke concentrations. These, together with low temperature and high relative humidity, are suitable for fog production[57] and made perfect by the pres-

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ence of sulphur trioxide and any other chemical fog producers[5]. A thick blanket is laid which permits even less of the sun's heat to reach the earth and something of a vicious circle is established. Locate all these conditions in a highly industrialized river valley such as that of the Meuse or the Monongahela, and the stage is set for a disaster which sooner or later occurs.

The influence of rain and snow on air pollution is subject to difference of opinion. Some investigators say that both tend to clear the air, and others that neither has appreciable effect. These differences in viewpoint may be accounted for by the fact that both deposit gauge readings and lead peroxide sulphur measurements are markedly influenced by rain. The Leicester survey[20a] concludes that their results "strongly suggest that rain plays a part in clearing the air of smoke and sulphur dioxide. No meteorological variable has so far been found which strongly affects the concentration of one form of suspended pollution while leaving the other unchanged." But in some places, at least, the part played by rain is not very great.

Willett[93], who has presented an admirable description of the influence of meteorological and topographical factors on air pollution, goes on to show how this knowledge may be used in controlling the problem. He suggests that an evaluation of these factors in any area will give a good indication of the potential danger of high concentrations of smoke. Use is now being made of these factors in the siting of industrial plants. The availability of labor and raw materials may outweigh the disadvantages of high levels of smoke pollution, but at least the matter must have consideration.

The last recommendation of the United States Public Health Service in its report on the Donora episode[78] urges the use of weather forecasting so that the community may be forewarned of adverse conditions and take appropriate action. Stanford Research Institute[83a] has, after a careful study of the pollution problem in Los Angeles, established a mathematical relationship between smog and the characteristic meteorological conditions associated with it. The Stanford Research Institute Smog Index is calculated from measurements of wind movement, visibility, relative humidity, mean temperature, and the height of the temperature inversion level. They claim considerable accuracy in their forecasting of smog with the use of this index.

Smoke and Man's Environment. The influence which smoke exerts on man's environment appears to be overwhelmingly detrimental. The sole possible exception is its weak bactericidal action[39]. Even this effect is neutralized to some degree by the shield it provides for organisms against the lethal effect of sunlight. This interference with sunlight has claimed the attention of many investigators[87].

Drinker and others[23] recorded hours of daylight in Boston in 1928–1929 and attempted to correlate the data with dust counts, wind velocity, and relative humidity. They concluded that loss of sunlight seemed less due to smoke than to natural causes. Barrett[7], in Toronto, showed that smoke could so reduce the ultraviolet light as to render it no longer capable of preventing rickets in albino rats fed on a rachitic diet. These findings were later confirmed by Kay and Barrett[47] in greater detail. The dosage and wavelength of ultraviolet light necessary to prevent rickets in rats was determined and incidental evidence was obtained to show that the proportion of ultraviolet light of different wavelengths transmitted by smoke is approximately constant.

A thorough investigation of the problem was carried out by Ives[42] on Man-

hattan Island. He found that relatively the greatest loss of light occurred in July and the least in December. The mean loss for the whole investigation was 21.5% but less on clear days and more on cloudy days. He concluded that loss of light depends on the altitude of the sun and the nature of the daylight and is independent of absolute humidity. The loss is proportional to relative humidity and inversely proportional to wind velocity.

The biological component of environment has also received much attention. Largely as a result of the damage done to crops in the valley of the Columbia river by sulphur dioxide emitted by a huge smelter at Trail, British Columbia, the part played by this substance in plant ecology has been thoroughly evaluated. Full information is to be found in 2 comprehensive papers by Katz[46a,46b] and one by Whitby[91]. It is clear that sulphur dioxide in concentrations up to 0.5 p.p.m. is not harmful to plants and may even be beneficial to those that are sulphur deficient. Higher concentrations are capable of causing considerable retardation in growth, especially after transplantation and when physiological activity is great. A great deal of circumstantial evidence of the deleterious effect on plant life of smoke, rather than sulphur dioxide, exists[44,62], but has not been so well quantitated.

The influence of smoke on animals has received little study apart from what can be deduced from the experimental studies on laboratory animals described below. In the Meuse Valley disaster many cattle had to be slaughtered[30], but at Donora[78] the effect on farm animals was reported as minimal. Among domestic animals there appears to have been a real increase in the morbidity and mortality rates attributable to the smog, especially among dogs. It is interesting that among dogs of known age the greatest mortality was experienced by those over 11 years and under 1 year, but dogs of known age are a select sample from which it is dangerous to draw inferences.

Damage by air pollution to the material objects of man's socio-economic environment is considerable and has received a full description by Shaw and Owens[80], and by Marsh[62]. There is erosion of stone and of metal, in which the acids of sulphur play the leading part. Buildings are blackened and works of art disfigured by soot and the solid products of combustion. Homes, furnishings, and clothing are made filthy and have their useful lives shortened by the increased cleaning which this necessitates.

What is the significance to man of all this damage to his environment? The answer is indeed hard to find and possibly it cannot be defined with any accuracy. It might be possible to evaluate the damage done to such things as buildings and bridges and the cost of fuel wasted and even the loss of productivity of land. But how is a price to be put on filth and the work it entails, or on the nuisance, the waste of time, and the physical danger it brings through impaired visibility to pilots of airplanes, drivers of automobiles, and the general population?

Smoke and Human Health. Early attempts to evaluate the influence of smoke pollution on human health were handicapped by lack of a good index of pollution. Observers drew attention to the fact that periods of severe fog were accompanied by respiratory death rates above what could reasonably be expected for the time of the year. There are several such occurrences on record: London in 1880[73a,73b], 1892[73a], and 1948[56]; Glasgow in 1909[94b] and 1925[70,73a,94b]; the Meuse Valley in 1930; and Donora in 1948. Of the same type was the observation made in Dublin during the winter of 1941[54b] when, owing to the coal shortage, at-

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mospheric pollution was half its usual level and the respiratory death rate was the lowest in many years.

All these events had certain features in common. They occurred in winter, the weather was cold, and most of the deaths were of people over 60 years of age, and frequently chronic sufferers from asthma, bronchitis, or cardiac disease. The association of respiratory disease with cold weather is even more obvious than that with fog. The seasonal swing in respiratory mortality takes place whether atmospheric pollution is present or not. The death rates in Dublin in the winter of 1941 were still well above the summer mean. William Farr described a weekly excess of 581 deaths in England and Wales during a period of exceptional cold in the winter of 1874[26]. Five years previously he had commented on the rising trend in the pneumonia and bronchitis death rates in Great Britain during the 19th century[94b]. He ascribed this to cold and the increase in industrial dust and smoke. Greenwood is reported by Russell[73b] as having found respiratory mortality more highly correlated with the temperature of the previous week than with the current weekly record. The relative importance of certain meteorological factors, temperature, rainfall, and relative humidity in the etiology of bronchitis and pneumonia was the subject of statistical investigation by Young[95] and Woods[94a,94b] in the 1920's. From their work it seems reasonable to conclude that climatic factors are relatively less important than other environmental factors in the etiology of respiratory disease in children under 5 than they are in adults, and especially people over 60 years of age. Of the climatic factors, temperature of the preceding week is the most important.

In 1923, Collis[16] in Britain made a careful statistical study of respiratory disease in relation to occupation. He produced evidence to suggest that by virtue of its behavior, bronchitis is a disease entity separate from pneumonia and phthisis. The bronchitis mortality was not closely related to occupation except insofar as there was exposure to an irritant gas or dust or to severe climatic conditions. The records appeared more compatible with a chronic traumatic than an infective origin for the disease. The work of Brundage and others[12] on pneumonia in the American steel industry suggested an association between this disease and exposure to wide temperature variations, inclement weather, and possibly the presence of sulphur dioxide. These two studies illustrate the different conceptions of the term bronchitis and pneumonia which exist on the two sides of the Atlantic.

Attempts to disentangle the effects of cold and of smoke cannot be said to have been very successful. In 1924 and again in 1926, Russell[73a,73b] compared the frequency of foggy days in 2 London boroughs with mortality rates from respiratory disease in adults over 55 and children under 5, over a period of 30 years. He concluded that temperature was more important than fog, but if fog is associated with low temperature, the death rate goes up in adults but not in children.

Shaw and Owens[80] questioned Russell's findings on the ground that fog and smoke are not synonymous and that the influence of smoke pollution may have been masked by the inclusion of simple water fogs in the total of foggy days. Clearly, foggy days are not a satisfactory measure of air pollution and other observers have used the next most simple index, soot fall.

Ascher[3] was one of the first to draw a parallel between the influence of coal dust on miners and the influence of particulate products of combustion on the general population, but he jumped to conclusions in deducing that smoke

is necessarily the cause of the increased respiratory disease of cities[52]. In 1913, White and Marcy[92] found a close correlation between respiratory mortality and soot fall in various parts of Pittsburgh, according to wards. They claimed that division of the city in this way ruled out a number of factors such as poverty, race, and congestion of population. Unfortunately, it is not likely that it also corrected for age and sex distribution, for which no allowance was made. In 1914, Klotz[51] produced autopsy evidence of the association of the pigmentation of lungs with city dwelling. He expressed the opinion that this pulmonary anthracosis results in decreased elasticity of the lungs and an increased fibrotic reaction to tuberculosis.

Schnurer[74b,75] and Haythorn and Meller[38] studied between them 3,000 autopsy records from Pittsburgh hospitals in 1937–1938. Their aim was to compare the distribution pattern of respiratory disease with that of pulmonary fibrosis due to extraneous pigment. They recognized the selected nature of the sample and the fact that the dust had many origins other than atmospheric smoke pollution. Their findings were equivocal and the conclusion to be had from their results, that the effect of soft coal smoke and dust on lungs is not entirely negligible, is about as much as seems justifiable.

No investigator has made greater use of soot fall measurements than Mills, who has repeatedly demonstrated that the rate of deposit in various geographical divisions of many American cities is correlated with the death rates from respiratory disease in the same areas[65a,65b,65c,65d,66]. This is particularly true of the rates for males, which are sometimes twice as high as for females in smoky areas. This fact is taken as evidence that socio-economic factors are not the main cause of increased mortality in highly smoke-polluted areas because this should influence males and females equally. It is suggested that males fare worse because they are more exposed to the atmosphere than females. The evidence presented by Mills is gravely weakened by the fact that no correction is made for the many possible environmental factors, of which one of the most important is the influence of occupation. This might well account for the sex differences upon which so much of the case rests. Furthermore, deductions based on mortality experience uncorrected for age distribution can never be conclusive.

In spite of what atmospheric sulphur dioxide can do to stone, there is little sound evidence of substantial damage by this gas to the human respiratory tract. Perhaps the most extensive study of this subject was by Kehoe and others[48] in 1932, who carried out a survey of men exposed for periods up to 12 years to known concentrations of sulphur dioxide in a refrigerator works. Some evidence of chronic irritation of the upper respiratory tract was found, though there were no significant radiographic changes. The influence of this damage on their future health could not be judged and it is unlikely that men unduly susceptible would remain long enough in the industry to demonstrate any chronic effects.

A roentgenologic survey of men exposed to sulphur dioxide in the refrigerator industry was carried out by Evans[28] and also showed no evidence of damage. Gardner[32] made a study of respiratory irritants, including sulphur dioxide, and found no evidence that exposure necessarily aggravates pulmonary tuberculosis or increases susceptibility to the disease. Some evidence does exist that sulphur dioxide is capable of precipitating asthma in a predisposed individual[17,72]. Many feel that the gas played at least some part

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in the Donora and Meuse episodes, but the evidence is circumstantial and in the Meuse at least, there is as much evidence against fluorine[71b]. Experiments in Los Angeles showed that sulphur dioxide could cause the conjunctival irritation so troublesome in that city, but so, too, could many of the other irritants which have been tested[83a,83b].

The carcinogenic power of soot, tar, and other products of combustion and destructive distillation of coal is now well established and it is natural that these products which also pollute the air should be suspected of a causative role in respiratory cancer[40]. Kennaway and Kennaway[49] brought evidence to show that there is a real increase in this disease in Britain, and that no correlation with occupation exists except to a slight extent with those who are exposed to tarred road dust. Although the incidence is higher in men than in women, and in urban than in rural areas, the rates of increase are much the same in both. A study of the incidence of cancer in the American carbon black industry was made by Ingalls[41]. In spite of heavy exposure to the soot from the incomplete combustion of natural gas and oil, workers in this industry did not experience unduly high mortality or morbidity rates from cancer. Stocks[85] made a study of regional differences in cancer mortality in Britain and found that cancer of the respiratory tract was significantly correlated geographically with hours of sunlight. Pulmonary tuberculosis and bronchitis showed a similar distribution, but not cancer of the stomach and of other sites. Stocks suggests that this may either be due to atmospheric pollution or to some inhibiting reaction of sun on respiratory cancer. No satisfactory evidence appears to exist for associating smoke pollution any more closely than this.

Carbon monoxide must be considered as a potential agent of disease associated with smoke pollution, though it seldom reaches high concentrations except in confined places. An investigation of police officers stationed in the Holland Tunnel was made by Sievers and others[81]. The men were exposed for prolonged periods, up to 13 years, to concentrations of up to 100 p.p.m. of carbon monoxide. No indication was found of adverse influence on their health. The problem of chronic carbon monoxide poisoning is examined at length by Grut[35]. It seems clear from his evidence that though carbon monoxide may be a hazard to certain occupational groups, and though any inhabitant of a big city is exposed to it almost daily, "this exposure does not amount to much".

So far, only the direct effects of smoke pollution have been considered. Indirect effects produced by the influence of smoke on man's environment are potentially many, but may be quickly disposed of because they have never been measured. The psychological effects of gloom[15] may be real, and there is no doubt of the fatigue of the housewife in a smoky atmosphere. The cost to health of loss of sunshine and ultraviolet light may be considerable, but has never been assessed satisfactorily.

Experimental Studies. Relatively few laboratory studies have been made relevant to the influence of atmospheric smoke on health. Klotz[51] attributes the first animal experiments in this field to Knauff in 1867 and Konradi in 1869. Haythorn[37] in 1913 published conclusions based on necropsy observations and on guinea pigs exposed to smoke. He considered that a moderate degree of anthracosis appears to interfere with normal free lymphatic drainage of the lungs and so renders the lower respiratory tract less able to deal with acute infections. On the other hand, it appears to encourage fibrosis and to aid the healing of tuberculosis.

Several attempts subsequently were made to determine whether or not coal dust or its products of combustion are capable of causing any degree of pulmonary fibrosis. Animal experiments with guinea pigs and rats and mice by Kettle[50], Haynes[36], Belt and King[9] and Campbell[13d] all suggest that coal dust and even carbon alone is capable of producing slight foreign body reaction in the lungs of these animals with slight resultant fibrosis of a reticular type. In general, the higher the silica content, the more the fibrosis, but there is no simple relationship and severe fibrosis may even depend on the presence of the tubercle bacillus[8].

Schnurer[74a] made the most direct attack on the problem by exposing rabbits, rats, and mice for a period of 80 days under controlled conditions to the flue gases from anthracite, coke, and bituminous coal. The constituents of smoke, though in normal proportions, were acting in much higher concentration than is ever found in the atmosphere. Pathological changes which took the form of bronchitis, pneumonia, pulmonary fibrosis, and failure to gain weight were considerably more severe in the bituminous group. It is interesting that the dust counts in the smoke from bituminous coal were about 12 times greater than from either of the other fuels, while the sulphur dioxide concentration from the coal and the coke were about 4 times that from the anthracite. This suggests that particulate matter played a big part in the production of disease in these animals, though not excluding contributory action by some other agent, including sulphur dioxide. A similar experiment by Schnurer and Haythorn[76] on rabbits and rats produced similar findings. This time, temperature and humidity were checked and steps taken to evaluate the role of silica in the results. Lung sections which showed characteristic inflammatory changes, fibrosis, and emphysema were incinerated and treated with hydrochloric acid. Microscopic examination by polarized light revealed no silica. Few animals were used in this experiment and it seems dangerous to draw any firm conclusions from it.

Campbell[13a,13b,13c,13d] investigated the power of certain dusts to produce primary tumors in mice. He found a significant increase in primary lung tumors, some of which were malignant and accompanied by metastases, following prolonged exposure to dust from tarred roads, and to a lesser degree to dust free from tar, and to soot from the exhaust of an internal combustion engine burning heavy oil. He failed to produce any increase with exhaust gases from internal combustion engines, tobacco smoke, chimney soot, anthracite dust, and bituminous coal dust.

Seelig and Benignus[79] showed that, with heavy dosage indeed, soot from Kentucky bituminous coal will produce in mice an 8% incidence of cancer of the lung compared with 2% in a control group. Animals of the experimental group were given soot instead of sawdust for their bedding and this was shaken up 2 and 3 times a day. The duration of this exposure was until death. Not even the most ardent campaigner against smoke would maintain that atmospheric pollution of cities is as bad as that.

The toxicity of sulphur dioxide was well investigated by Weedon, Hartzell, and Setterstrom[90]. Various species of animals were exposed for periods up to 30 days to varied concentrations of the gas. Male guinea pigs and mice showed no changes whatever to less than 10 p.p.m. and only slight pulmonary and gall bladder changes to less than 33 p.p.m. Above this level toxicity increased, with production of corneal opacities, gastric ulcers and massive pulmonary hemorrhages, and

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death. Intermittent dosage was associated with less toxicity. From this evidence, the conclusion was reached that sulphur dioxide by itself is unlikely to have any influence on human health in even the worst conditions of atmospheric pollution. It was admitted, however, that the co-existence of dust and infection might change the picture.

An interesting contribution was made by the work of Cralley[18], who studied the effect of sulphur dioxide on the ciliary activity of excised rabbit's trachea. Ten minutes' exposure to 30 p.p.m. abolished ciliary action, and it was suggested that smaller concentrations for a longer time might impair ciliary activity.

The oxidation of sulphur dioxide to sulphur trioxide in the atmosphere raises the question of the toxicity of the latter compound. This has been the subject of recent study by Mathur[63] and Treon and others[88] who have exposed animals to sulphuric acid mist and found that guinea pigs were more susceptible than mice to its effects and that its toxicity seemed appreciably greater than that of sulphur dioxide. Further investigation by Amdur[2] of the effects of this agent on guinea pigs suggests that the age of the animal and the duration of exposure are important variables. The concentration of sulphur trioxide sufficient to kill 50% of the guinea pigs when exposed for eight hours was 5 p.p.m. for those 4 to 6 weeks old, and 12.5 p.p.m. for those over 18 months. Below 2 p.p.m. no apparent damage was done by even 72 hours of exposure.

The frequent incrimination of hydrofluoric acid by certain writers as the agent of disease in the Meuse and Donora episodes justifies the inclusion here of the experimental studies by Machle and others[58,59,60] on the toxicity of this gas to animals in various concentrations. The evidence offered by these workers suggests that, in concentrations below the level where acute irritation could be appreciated, prolonged exposure produces chronic pulmonary and systemic changes in rabbits and guinea pigs. It is not clear whether this level is ever reached in any smoke polluted atmosphere.

An appraisal of Evidence. No matter how well measured and controlled the evidence bearing on a problem may be, its final evaluation depends on the instinctive conceptions of probability brought to bear by the evaluer. This is largely a function of past experience. No suggestion is intended against striving constantly to obtain mathematical statements of probability with their limits of possible error. What is said is indeed in vindication of such efforts, which aim at reducing the personal factor to a minimum. It is justification, nevertheless, for the attempt now made with the meagre and often insufficiently controlled evidence that exists, to evaluate by reasoning, logic, and experience the direct influence of atmospheric smoke pollution on human health.

As a potential agent of disease, smoke consists of a mixture of particulate and gaseous matter, constantly varying in concentration and in physical and chemical composition. The factors of importance seem to be soot particles and possibly their silica and tar content, the sulphur dioxide–trioxide complex, fluoride, and total acidity. Any influence these factors may have depends on their quantitative and qualitative characteristics while in contact with human tissue, and we are decidedly short of such measurements. Although tolerably accurate measurements may be at hand, of how much soot settles on the roof of the Town Hall, we know next to nothing of the size, concentration, and composition of the particles which reach the respiratory tract of men in the street. Certainly, the variations in concentration

from hour to hour and from season to season are so great that mean values over a long period of time give doubtful indication of what we really want to know. We do know, however, that the dosage of the agent at work in any community is determined by the nature and quantity of fuel, methods of combustion, topography, and meteorological factors, and it might even be possible to obtain its measure in these terms.

The case against smoke stands or falls by its effect on the respiratory tract. That is where we would expect its results, and that is where diseases of the sort we would expect do occur. The strongest evidence is probably supplied by the episodes of association between severe fog and high death rates from respiratory disease, but so far we have no way of separating the influence of a period of high concentration of smoke from the influence of the environment which determines that concentration. The most important of these environmental factors are probably temperature, atmospheric pressure, and humidity. The irritant action of smog on the throat is a subjective phenomenon appreciated by most people who have ever experienced it. It seems unlikely indeed that the smoke content of fog plays no part whatever in producing the illness and deaths which accompany it; and it seems probable that it plays a major part.

If it is admitted that severe smoke pollution can be one of the causes of death from respiratory disease, then it follows reasonably that lesser degrees of pollution are likely to cause pathological results of less severity. Individual variation in susceptibility must also be taken into consideration. Though it seems clear that old and debilitated persons are most susceptible, nothing whatever is known of whether recurrent subjection to doses of smog increases or decreases resistance to it. Nor do we know whether the phenomena of allergy and hypersensitivity contribute to the problem.

It is often pointed out that the highest concentration of any irritant gas ever encountered in the atmosphere is much lower than that required to do any harm to animals or man under controlled conditions. It should be remembered, however, that multiple causation is the rule in natural disease, and any search for a single responsible agent of itself alone responsible for a morbid process is likely to be doomed to failure. Smoke itself is a mixture of many potential agents and it seems reasonable to expect some degree of synergistic action both between them and between infective and climatic factors. Nevertheless, the order of toxicity of these agents does suggest that damage to respiratory tissue by the irritant gases is liable to be confined to relatively rare occasions when pollution concentrations are exceptionally high. The Meuse and Donora records point to duration of exposure as a very important factor. The difficulty is to fix a point from which to measure exposure, but most of the severe illnesses in both episodes seemed to have their onset after 2 to 3 days of dense fog.

Particulate matter presents a different problem. Such material is undoubtedly stored in the lungs of city dwellers, and chronic and cumulative effects must be considered. The particulate matter of smoke is relatively inert, but much evidence suggests that it is not completely so. Some foreign body reaction occurs, with minor degrees of reticular fibrosis and interference with nomal lymphatic drainage. In normal health, such changes are not detrimental to the individual, but do lungs modified in this way react to infective and physical agents as efficiently as those not so affected? We do not know the answer to this ques-

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tion, but it seems possible that their response to acute infections and irritants may be impaired, and to the tubercle bacillus is more than usually fibrotic.

The carcinogenic activity of atmospheric smoke must be left as an open question. It seems fairly certain that soot can produce cancer, but only under special circumstances and in dosages far greater than anything ever supplied by atmospheric smoke. The problem is further complicated by smoke from quite another source, namely tobacco. Recent investigation by Doll and Hill[21] and Levin, Goldstein, and Gerhardt[55] suggest that definite association exists between cigarette smoking and cancer of the lung. The evidence against smoking is certainly much stronger than any presented against atmospheric smoke, but once again the possibility of synergism cannot be excluded. The geographic studies of Stocks[85] and Meyers[64a,64b] are sufficiently suggestive to justify further investigation of this problem.

The Direction of Future Studies. In the further evaluation of smoke as an agent of disease, certain lines of investigation suggest themselves within the field of epidemiology. Firstly, the best possible use is to be made of available data. Past efforts have been inconclusive because insufficient care has been taken to exclude such factors as age and sex distribution of the population at risk, temperature and relative humidity, and socio-economic factors. Indices of both health and pollution need to be carefully chosen to ensure adequate comparability in time and place. Sulphur dioxide measurements might well be a better index of the pollution to which men are actually exposed than is sootfall; and thus far little, if any, use has been made of them. Studies of this sort need to make more use of statistical techniques to determine the importance of multiple variables.

Secondly, laboratory studies on animals should be made to assess the important question of synergism and antagonism among components of smoke, infective agents, temperature, and relative humidity in their action upon respiratory tissue.

Lastly, field studies of specific population groups would yield information on the range of variation of susceptibility of human beings to the effect of certain irritants and of factors which influence it. Examination of the health records of some group such as postmen, whose socio-economic circumstances are roughly constant in different parts of the country, seems to offer the best possibility of correlating health in different cities with levels of smoke pollution, for which comparable measurements exist.

The need for further study of the many aspects of the problem of atmospheric smoke pollution is clear and has been urged by many writers on the subject in recent years[4,10,22,25,78]. Their recommendations cover a wide range and it is hoped that this epidemiologic review will support the need for consideration of the problem in terms of man's total ecology, to which end the contributions of many sciences are needed.

The significance of atmospheric smoke pollution is to be found by weighing the damage done against any associated benefits and the cost and efficiency of any control measures. The balance may be struck in a variety of units—dollars, man hours, death or disease—and preferably in as many as are feasible, because it is not easy to establish their "rate of exchange". Initially, some of the estimates may be appallingly crude and rough and possess a wide range of possible error, but only through increasingly refined estimates of total cost can a sound evaluation of smoke control, or of any other public health activity, be made.

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PATHOLOGY AND BACTERIOLOGY

UNDER THE CHARGE OF

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LISTERIA MONOCYTOGENES AS THE CAUSE OF DISEASE IN MAN AND ANIMALS, AND ITS RELATION TO INFECTIOUS MONONUCLEOSIS FROM AN ETIOLOGICAL AND IMMUNOLOGICAL ASPECT

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IT seems fitting to review briefly the abundant and diverse information that has accumulated over the past 25 years regarding *Listeria monocytogenes* as the definite cause of a specific disease, listeriosis, together with a consideration of infectious mononucleosis (glandular fever), a disease of somewhat uncertain etiology at the present time. Many investigators have attempted to establish some sort of a relationship between these two entities. Although they may be completely unrelated, certain evidence is outstanding which deserves elaboration before we discard Listeria completely in favor of the more popular view of a completely viral etiology for infectious mononucleosis.

I. **Listeria monocytogenes as the Cause of Diseases in Man and Animals.** Listeriosis is a sporadic, infectious disease occurring naturally in man and in many other animal species. The disease is caused by *Listeria monocytogenes*, a small Gram-positive motile rod, averaging 1 to 2 microns in length and about 0.6 micron in diameter. It is not a notably fastidious organism, growing freely on the usual laboratory media, but it has restricted fermentative power. It has a characteristic growth in semi-solid gelatin–agar. On blood agar, it produces small, faintly opalescent, transparent, almost pinpoint colonies at 24 hours, and after 48 hours hemolysis can usually be seen. The organism produces a monocytosis when a well regulated dose is injected into rabbits, which is readily noticeable after 48 to 72 hours. The proper selection of dosage is important since our own observations have shown that quantities as little as 0.25 cc. of a 24-hour glucose broth culture of some strains are fatal in less than 24 hours[22a]. The monocyte count in the blood of experimentally and naturally infected

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rabbits has been found to rise as high as 20,000 per c mm. and is the most striking character of this species. A second characteristic manifestation of experimental infection is the curious acute ophthalmia in rabbits and guinea pigs.

Anton[1], in 1934, showed that the organism is capable of producing a severe conjunctivitis, and later keratitis, in guinea pigs by swabbing the everted eyelid with culture material or by simple instillation into the conjunctival sac. At about the same time, Pons and Julianelle[50] found that the eye reaction in rabbits and guinea pigs was absolutely specific and were even able to produce the reaction in animals immunized against the whole organism. Julianelle[32] later surveyed a great number of organisms for this property and found only two which could produce it: *Listeria* and *Erysipelothrix*. The latter, however, gave the reaction only occasionally, but when it did, death of the animal invariably occurred, much in contrast to regular recovery of animals with the Listeria-produced ocular reaction. Furthermore, all the strains of *Listeria monocytogenes* examined produced the reaction.

Listeria monocytogenes was first isolated and identified by Murray, Webb and Swann[43] in 1924 from an epizoötic among laboratory animals at Cambridge. In 1929, Nyfeldt[45] recovered the organism from the blood stream of a boy with infectious mononucleosis, this being the first recognized isolation of *Listeria monocytogenes* from a human case. The organism was first recognized as the cause of disease in domestic animals by Gill[19] in 1931, when he isolated it from sheep in New Zealand and gave the name "Circling Disease" to the encephalitis which it caused. *Listeria monocytogenes* has been isolated from approximately 13 different species of mammalian hosts in at least 15 different countries; most of these are listed in articles by Graham *et al.*[22b] in 1943 and Kaplan[35] in 1945. Kaplan[35] gave the mortality in human listeriosis in its typical meningeal form as 68%. This figure he obtained from a survey of reports up to 1945 and he added that if recent cases treated with the sulfonamides were excluded, this figure would be 79%. Stanley[60] isolated a strain of *Listeria monocytogenes* from a fatal case of meningitis recently and claimed that this strain is 20 times less sensitive to penicillin than a sensitive strain of *Staphylococcus aureus*. Our own experience has shown that this same strain* shows only a moderate sensitivity to streptomycin[22a]. Gray, Stafseth, and Thorp[23] reported that the strain rapidly acquires resistance to streptomycin.

Probably the first real survey of the whole group was undertaken by Paterson[47] in 1940, who studied 54 strains of *Listeria monocytogenes*. He concluded on the basis of his findings that there is no relationship between the zoölogical host species, man or animal, and the bacteriological type; nor do the types appear to be associated with a particular geographic origin. It is also of interest that all the 9 strains studied, isolated from human cases of infectious mononucleosis, appear in a distinct group, No. 3 of his classification of 4 antigenic groups. These groups he determined on the basis of somatic and flagellar agglutination and absorption of agglutinins. In any work involving differentiation of strains by antigenic characteristics, the technique should be carefully controlled in the light of the findings of Paterson[47], Robbins and Griffin[51], and Hutner[27].

The clinical symptoms and pathological lesions associated with Listeria

* We are indebted to Dr. N. F. Stanley of Prince Henry Hospital, Institute of Epidemiology and Preventative Medicine, Sydney, Australia, for this strain.

infection seem to vary with the species of the host. In the rabbit, guinea pig, and gerbille, a generalized infection associated with necrotic liver foci is characteristic. In the chicken, according to Seastone[56], the disease is also generalized, and massive necrosis of the myocardium usually occurs. In ruminants the natural disease is manifested by an encephalitic or meningitic infection, and only through experimental infection is it possible to produce in them the systemic disease found in rodents[22b].

Very little is known as yet regarding the epizoology of listeriosis. Observations at the University of Illinois Agricultural Experiment Station[22b] indicate that "in ruminants the disease does not ordinarily sweep through a herd and affect a high percentage of animals, but rather runs a more leisurely course, usually involving less than 10% of a herd over a period of several months. While the morbidity within the herd is thus relatively low, the mortality in infected animals is very high."

Although the incidence of human listeriosis also indicates a low degree of communicability, 2 case reports are worthy of mention. Burn[10] reported 2 infants born the same day in the visiting obstetric service of a hospital. One infant, delivered early in the morning, died of Listeria meningitis the next day. The second infant, apparently delivered later on the same day, suddenly became ill 8 days later and died of the same cause within 6 days. Atkinson's[2] 5 cases in children all occurred within a period of 13 days, and 4 out of 5 were fatal. This would indicate that listeriosis is capable of assuming epidemic proportions when favorable circumstances are present.

Concerning the reservoir of infection and the mode or modes of transmission, little information is available. That there is some unrecognized factor in natural infection is suggested by the difficulty with which the disease is produced experimentally. Graham *et al.*[22b] feel that the ruminant and the rodent may not be the usual hosts of the organism, since listeriosis enjoys such a wide and sporadic occurrence. Gill[21] suggested that the sheep nasal fly, *Oestrus ovis*, might be concerned in the transmission of ovine listeriosis, but he was unable to support this hypothesis with experimental evidence, and the absence of larvae in some of the affected sheep seems to be evidence in itself against this suggestion. Olafson[46] felt that the rat may be a carrier since the appearance and disappearance of the disease in cattle barned-up for the winter months seemed over a period of years to coincide with the appearance and disappearance of the rats in these barns. *Listeria monocytogenes* has been isolated from rats, themselves suffering from the disease, by Machiavello[41] in Northeastern Brazil, so the association here may be real. One experiment was attempted by Olafson[46] along this line but failed to reveal any connection. Bigland[8] has reported a canary isolation of *Listeria monocytogenes* wherein several mites, *Dermanyssus gallinae*, were found on the body of the bird, but apparently no attempt was made to cultivate the organism from these parasites.

Recently, *Listeria monocytogenes* was isolated from ferrets at the U. S. Army Medical School[42], the animals apparently not suffering from the disease. This finding would seem to pose the question of healthy carriers in the epidemiology of this disease. In this connection, Stanley[63] examined a total of 3558 swabbings taken from the human nose, throat, or nasopharynx. Fifteen of these cultures were agglutinated by listeria antiserum above that of normal rabbit serum; two of these showed morphological, cultural, and biochemical properties similar to *Listeria monocytogenes*. Neither cul-

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ture, however, invoked the monocytic response nor the ocular reaction. This appears to be the first large scale attempt to establish some sort of human carrier incidence of *Listeria monocytogenes*. A selective and enrichment technique for the cultivation of Listeria has been brought forth by Gray *et al.*[24] and may prove useful for future experiments along this line. These workers report excellent results with 0.05% potassium tellurite added to plain nutrient broth and used as an enrichment medium.

The avenue of Listeria infection in animals is equally vague, but many investigators feel that listeriosis begins as a rhinitis. This supposition is supported by the fact that experimental listeriosis, particularly in ruminants, similar in all its aspects to the natural disease, is not ordinarily produced by intravenous or subcutaneous inoculation[22b], whereas Gill in 1933[20] reported that repeatedly drenching the nostrils of sheep resulted in meningitis and encephalitis. Also, Pons and Julianelle[50] reported in 1939 that an organism which was definitely *Listeria monocytogenes* was isolated from the throat of a girl whose blood contained the organism. On the other hand, Julianelle[31] later reported that instilling or spraying the culture into the noses of rabbits and monkeys failed to produce the disease, while Murray[43] succeeded in infecting 3 baby rabbits by feeding the culture to them in place of drinking water. This observation would incriminate the gastrointestinal tract as a possible portal of entry. Obviously, further investigation is necessary to establish the avenue of infection and the mode or modes of transmission.

Graham *et al.*[22b] report that the sera of a relatively high percentage of cattle with no history of exposure to *Listeria monocytogenes* contain agglutinins for the organisms. It was also noted by Seastone[56] that this organism was agglutinated by normal horse serum. Whether the disease is widespread in animals that never show symptoms or whether the agglutinins present are nonspecific, such as those in tularemia antisera to Brucella organisms, is not clear. Nevertheless, the Illinois workers[22b] report that agglutination and complement-fixation techniques are for the most part unsatisfactory for early diagnosis of listeriosis in cattle and sheep. They made numerous attempts at the immunization of rabbits, guinea pigs, chickens, and sheep against listeriosis without encouraging results. It has been proposed by Julianelle[31] that the stimulated antibody is not protective, since he found that antisera of high agglutinating titer offered little or no protection in mice.

With human listeriosis, a precipitation test with extracted polysaccharide used by Drew in 1946[13] has proved helpful for diagnostic purposes and offers a simplified technique for the serological differentiation of *Listeria monocytogenes* into 2 antigenic groups.

Murray, Webb, and Swann[43] noted that in sections examined from rabbits and guinea pigs suffering from listeriosis, the bacilli were distributed in clumps in small or large areas of focal necrosis. In the lungs, liver, kidney, and spleen they were frequently found forming small bacillary emboli in the smaller vessels. From the mesenteric lymph glands and suprarenals pure cultures of the organisms were often found. Heart blood cultures, however, were frequently negative, and only when overwhelming doses were given did they become positive. It was noted that in the natural disease, negative heart blood cultures were the rule.

In ruminants and humans the lesions produced in the natural disease are largely limited to the central nervous system. Many different organs were cultured at the Illinois Agricultural Station[22b] from cattle and sheep suffer-

ing with the natural disease, but the organism was isolated only from the central nervous system and in the great majority of cases only from the medulla. These investigators described the syndrome here as being characterized by "perivascular infiltration with cells, among which lymphocytes and monocytes predominated. The distribution of lesions in the brain suggests the possibility of spread by way of the meninges, canal systems, and vessel sheaths."

Piric, in 1926[49], observed that liver lesions when present were discrete foci of necrosis and these were sometimes preceded by visible fatty changes in the liver cells. According to Webb and Barber[69], liver necrosis first appears in the hepatic cells surrounding Kupffer cells which have ingested numerous organisms. Stanley, in 1949[61], reported that a chloroform and ether-soluble lipid extracted from *Listeria monocytogenes* was responsible for the production of monocytosis in rabbits, and that this lipid monocytosis-producing-agent (MPA) is liberated chiefly in the liver. He was able to elicit the typical monocytic response with a Seitz-filtered saline extract of necrotic liver, whereas the normal liver did not produce this response nor did any of the other normal or infected organs. This lipid MPA was "per se" non-antigenic and relatively non-toxic for rabbits. This work has been confirmed by us and furthered to show that the lipid MPA, when combined with heterologous conveyor protein to effect the antigenicity of the former, is probably not responsible for the sheep erythrocyte agglutination commonly seen in infectious mononucleosis[22a]. Stanley, in a later report[62], using the same MPA extract, has demonstrated an interesting adjuvant action upon antibody production in rabbits which appears to be related to monocyte stimulation.

II. Infectious Mononucleosis from an Etiological and Immunological Aspect. The relationship of this organism to infectious mononucleosis is a highly controversial topic. Nyfeldt in 1929[45] isolated the organism from the blood stream of a patient apparently suffering from infectious mononucleosis. Later Schmidt and Nyfeldt in 1938[54] described 5 cases of infectious mononucleosis accompanied by meningoencephalitis, in 4 of which *Listeria monocytogenes* was found in the blood or cerebrospinal fluid. Pons and Juianelle in 1939[50] also isolated the same organism from the blood of such a patient, and Webb in 1943[68] reported a similar finding.

One of the most serious drawbacks in postulating any relationship between infectious mononucleosis in man and *Listeria monocytogenes* is the inability of the organism to stimulate the formation of sheep erythrocyte agglutinins in the rabbit. This observation has been reported many times and has been extended recently by Stanley[61] to show that the protein, polysaccharide, and lipid fractions prepared from a strain of *Listeria monocytogenes*, which he isolated from a fatal case of Listeria meningitis, could neither agglutinate sheep erythrocytes nor stimulate the production of sheep erythrocyte agglutinins in the rabbit. Of possible further significance is the fact that, in the cases of infectious mononucleosis which he studied, absorption of sera with Listeria suspensions did not reduce the sheep erythrocyte agglutination. Wising, in 1942[70], made a careful study of 27 typical cases of the disease, but neither from the blood in the acute febrile stage nor from the excised lymph nodes was he able to cultivate this organism. Convalescents from infectious mononucleosis contained no agglutinins in their blood to *Listeria monocytogenes*. Inoculation of killed *Listeria monocytogenes* into human volunteers did not give rise to a Paul–

Bunnell reaction; and inoculation of the living organisms into monkeys led to a rapidly fatal infection, accompanied by suppurative meningitis and encephalitis, which was unlike typical infectious mononucleosis in man. Janeway in 1941[28] studied 13 typical cases of infectious mononucleosis and reported that the Paul–Bunnell agglutination was uniformly positive in all cases; Listeria agglutination titers showed a slight elevation above those of a normal control group, but there was no significant trend upward or downward in the Listeria agglutinin titer during the course of the disease.

On the other hand, the results of Stanley[61], Webb[68], and Julianelle[30] are in agreement that in a certain number of cases of infectious mononucleosis (Julianelle places it as high as 46%) there is significant Listeria agglutination increasing in titer during the course of the disease and gradually falling off with the Paul–Bunnell titer. Webb[68] found that 6 out of 9 patients diagnosed as infectious mononucleosis gave agglutination titers to *Listeria monocytogenes* varying from 1/125 to 1/500, and in one case demonstrated a rise in titer from 1/25 to 1/125 over a period of 11 days. Stanley[61] speaks of another case wherein both the Paul–Bunnell titer and the Listeria agglutination titer increased over a period of 8 days: the Paul–Bunnell from 1/32 to 1/256 and the Listeria from 1/4 to 1/128. Through the use of various antigens prepared from the Salmonella, Proteus and Brucella groups, he minimizes the possibility of nonspecific stimulation of Listeria agglutinins in these people. In rabbits inoculated intraperitoneally with *Listeria monocytogenes*, in small enough dose to produce a non-fatal disease, the resulting antisera had usually a low titer, rarely exceeding 1/64. Human Listeria titers, if specific, thus assume an importance which should not be overlooked.

Of the 20 cases of infectious mononucleosis examined by Stanley[61], 13 gave positive Paul–Bunnell reactions after preliminary absorption of the sera with guinea pig kidney. Of these 13 cases, 7 or 35% gave significant Listeria agglutination, considerably above those values from normal serum controls.

Perhaps somewhat more impressive is the evidence which has accumlated to suggest that the disease is caused by a filterable virus having a predilection for lymphatic tissue. Van den Berghe and Liessens in 1939[5] and van den Berghe, Liessens, and Kovacs in 1939[6] were able to produce a circulating monocytosis in a monkey by intramuscular inoculation of 4 cc. of oxalated blood from a child suffering from infectious mononucleosis, and were able to reproduce a similar result in a second monkey inoculated with blood from the first monkey, after it had been hemolyzed and filtered through a Seitz EK filter disc. The virus was grown in tissue culture, and still proved capable after 10 generations of causing monocytosis in monkeys. Mice, however, inoculated intracerebrally proved insusceptible.

Verlinde *et al.* in 1950[67] reported successful transmission of infectious mononucleosis to Cynomologous monkeys using bacteriologically sterile blood, suspensions of swollen lymph glands, and throat-washings treated with penicillin and streptomycin, all taken from patients suffering from infectious mononucleosis. The principal features of the experimental disease were a rise in the Paul–Bunnell titer and an increase of the percentage of mononuclear cells, a slight leukocytosis succeeded by slight to moderate leukopenia, and sometimes a febrile reaction. Worthy of mention is the fact that in one monkey there was a rise in the Paul–Bunnell titer from 1/32 to 1/4096 over a 36-day period, along with an abnormally high percentage of

mononuclear cells. Citrated blood (5 cc.) from this monkey inoculated intramuscularly into another monkey produced a sharp rise in Paul–Bunnell titer after 8 days.

In 1942, Nettleship[44] described the production of pearl-grey nodules with heavy monocytic infiltration in the chorio-allantoic membrane of chick embryos inoculated with Berkefeld filtrates of nasal washings or with blood of infectious mononucleosis patients.

Sohier, Lepine, and Sautter[59] in 1940 reported a successful human transmission using whole blood obtained from a patient with infectious mononucleosis. The subject was injected intramuscularly with 6 cc. of this inoculum and subsequently developed suggestive hematological and serological characteristics of the disease even though he had few symptoms and no fever.*

Wising in 1942[70] failed to produce the disease in rabbits inoculated intravenously with heparinized blood of febrile patients diagnosed as cases of infectious mononucleosis but had some success with monkeys. Of 7 Macacas monkeys inoculated intracerebrally, intraperitoneally, or subcutaneously with fresh lymph node suspensions from patients in the acute stage of the disease, 3 developed mild clinical symptoms, with general enlargement of the lymph nodes, coming on after 8, 17, and 18 days, respectively. There was also a slight monocytosis. These changes were reproduced on fresh monkeys by inoculation of excised lymph nodes; 2, 3 and 5 passages were made successfully with the 3 different strains. Histological examination of the nodes revealed the typical, irregular, pleomorphic, large-celled hyperplasia seen in man. Of 6 human volunteers inoculated with heparinized blood or plasma from patients in the acute stage, 5 remained well. The sixth who had received 250 cc. of whole blood developed infectious mononucleosis 18 days later. The characteristic blood picture showing large atypical lymphocytes was present here, but the highest Paul–Bunnell titer reached during the entire course of the disease was only 1/32 after preliminary absorption with guinea pig kidney.

Along with these apparently successful transmissions, numerous failures have been reported. In 1948, Evans[16] conducted 21 experiments attempting the transmission of infectious mononucleosis to human volunteers, using whole blood, blood serum, and unfiltered throat washings without successful transmission of the disease in a single instance. Others have reported similar findings[33].

The ability of the blood of infectious mononucleosis patients to agglutinate sheep red corpuscles was described by Paul and Bunnell in 1932[48]. Normal agglutinins, rarely exceeding a titer of 1/56, may be found in healthy persons, according to Smeall[58], and the heterophile antibody content may rise after injection of horse serum. Observations by Stuart and his colleagues in 1936[65], by Bailey and Raffel in 1935[3], Davidsohn in 1937[12], and Barrett in 1941[4] have shown that the normal heterophile antibody can be absorbed completely from the serum by means of guinea pig kidney, that that met with in serum disease can be absorbed by guinea pig kidney and ox red corpuscles, and that the heterophile antibody of infectious mononucleosis can be absorbed by ox red corpuscles but not by guinea pig kidney. Results of inhibitory and absorptive experiments carried out by Stuart *et al.* in 1936[66] agree in showing that the sheep erythrocyte agglutinins in infectious

* Since the writing of this article, R. Sohier has published a review which is complementary and should be consulted: Mononucléose Infectieuse, Encyclopedie Medico-Chirwrgiale, Paris, 1950.

mononucleosis are not Forssmann type antibodies, for they react with alcohol treated sheep and beef cells but not with the alcoholic extract of these cells.

Halcrow, Owen, and Rodger in 1943[25], who made a study of several cases of infectious mononucleosis, stated that a Paul–Bunnell titer of 1/32, especially when accompanied by preliminary absorption of the serum with guinea pig kidney, is highly suggestive of infection. Many investigators have reported, however, that all cases diagnosed as infectious mononucleosis do not give a positive Paul–Bunnell test. Shafar and Weir in 1943[57], for example, described an outbreak of infectious mononucleosis in Scotland, affecting about 200 persons, in which the Paul–Bunnell reaction was almost uniformly negative. MacFarlan and MacFarlane in 1943[40] likewise described an outbreak in a girls' school affecting 78 pupils, in which typical blood changes were present, but not a single serum reacted positively in the Paul–Bunnell test. According to Kaufmann in 1944[36], and Kruger in 1945[39], it is usual to find that approximately 30% of cases that are clinically and hematologically diagnosed as infectious mononucleosis give negative Paul–Bunnell tests. It is a pity tetration of antibodies to Listeria was neglected in this large senes of Paul-Bunnell negative cases.

Numerous reports of positive Wassermann tests have been made in cases of infectious mononucleosis[53]. Bernstein[7], for example, has reported that positive Wassermann tests were found in 15% of the cases he examined. Kampmeier[34] stated that some Japanese authors have reported an incidence of positive Wassermann reactions at some time in the course of the disease in as many as 40% of cases. He himself found that 6 out of 30 cases examined showed positive Wassermann reactions. Kolmer[38], using rabbits injected with living and heat killed strains of *Listeria monocytogenes*, was unable to demonstrate any complement-fixing or flocculating antibodies to antigens commonly employed in the serum diagnosis of syphilis.

In 1946, Burnet and Anderson[11] demonstrated the capacity of serum from some cases of infectious mononucleosis to agglutinate human 0 erythrocytes previously modified by absorption and subsequent elution of Newcastle Disease Virus (NCD). According to a recent report by Kilham[37], this capacity runs roughly parallel to the Paul–Bunnell agglutination. On the other hand, Verlinde *et al.*[67], after a similar survey, claimed that no correlation could be demonstrated between the titer of the Paul–Bunnell reaction and that of the NCD virus-treated red cells. They observed that a number of mononucleosis sera with a low Paul–Bunnell titer showed an increased NCD virus hemagglutination titer. This discrepancy should be resolved as soon as possible, since the latter observation may well have certain diagnostic value.

Anderson[18], in a study of 75 patients with infectious mononucleosis, found 52% presented an increased hemagglutination titer to NCD virus-treated human 0 cells; Evans[17] reported an incidence of 74.5% and Verlinde *et al.*[67] reported 70%. These values all rest on results with the Australian strain of NCD virus. Hemagglutination percentages were found to be considerably less when a Connecticut and a California strain of NCD virus were used for comparative purposes by Evans[17].

Different serum factors appear to be responsible for Paul–Bunnell agglutination and NCD virus-human 0 hemagglutination. Although infectious mononucleosis sera share with Newcastle disease immune sera the ability to agglutinate NCD-virus sensitized erythrocytes, the mononucleosis sera do

not inhibit hemagglutination by NCD virus[37]. Evans and Curnen[15] have shown that increased hemagglutination titers to NCD-sensitized erythrocytes are commonly observed in other infectious diseases such as lung tuberculosis, tuberculous meningitis, secondary syphilis, pneumonia, and infectious hepatitis. Kilham reports[37] it to be considerably increased in alcoholic cirrhosis of the liver. Thus the diagnostic value of the test seems to be proportionately reduced.

These and other observations have led some to believe that the hemagglutinin present in these cases is related in some way to hepatic dysfunction. Liver involvement has been shown to be of frequent occurrence in infectious mononucleosis[16,29,9] and Sterling[64] has shown, using electrophoresis, that the alpha, beta, and especially gamma globulin fractions are often elevated.

The findings related here thus far concerning proposed viral and bacterial etiology for this disease need not be opposed to one another. It is the opinion of many authors, Schultz in 1930[55] and Horing in 1933[26], that in dealing with so-called infectious mononucleosis in man we are not dealing with one disease but with 2 or more entities almost indistinguishable clinically and hematologically.

The hypothesis advanced by Stanley[61] is deserving of mention. He proposes a serological classification of infectious mononucleosis sera into 3 groups and gives the percentages as he encountered them in a study of 20 cases:

Group	*Paul–Bunnell*	*Listeria Agglutination*	%
A	positive	positive	35
B	positive	negative	35
C	negative	negative	30

He suggests that in his classification, Group A infections may be due to *Listeria monocytogenes*, while B and C infections would be attributable to a virus, and neither agent in turn responsible for the sheep erythrocyte agglutination. This view seems to be most in accord with the experimental findings to date, and explains his observation that absorption with sheep cells of infectious mononucleosis serum does not lower the Listeria agglutination, nor does absorption with suspensions of *Listeria monocytogenes* lower the Paul–Bunnell titer when the two occur simultaneously.

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PROCEEDINGS OF

The Physiological Society of Philadelphia

SESSION OF JANUARY 16, 1951

Production and Transfer of Myelogenous Leukemia in the Rat. HARRY SHAY, M. D., M. GRUENSTEIN, C. HARRIS, M. D., and L. GLAZER, (Fels Research Institute, Temple University Sch. of Med., Phila.). In experiments in which methylcholanthrene was instilled into the stomach of random-bred Wistar strain rats, three cases of myelogenous leukemia developed. Attempts to transfer the leukemia to 40-day old rats failed. Transfers were accomplished to animals younger than seven days. Two-tenths of a cubic centimeter of blood or of liver or spleen cells were injected intraperitoneally for transfer. Diagnosis of induced or transferred leukemia was always made first from peripheral blood and subsequently corroborated by bone-marrow smears and tissue sections. Average period of "take" 6–10 weeks. Occasional "takes" were at 5 weeks and sometimes as long as 5 months. White blood counts ranged from 50,000 to 292,000 with 7% to 30% of myelocytes. The leukemia was chronic in type and on transfer consistently resulted in a chloroleukemia with a histologic picture similar to that described by Wilens and Sproul (Amer. J. Path. **12**, 249, 1936) and by Oberling, Guerin and Guerin (Bull. de l'asso. française pour l'étude du cancer **28**, 214, 1939). To date, transfer by means of blood has been successful in our Wistar strain in 26 of 31 animals (84%), with liver tissue in 16 of 30 (53%), with spleen in 7 of 18 (39%). It also has been transferred by means of blood to 8 of 13 (62%) random-bred Sherman strain rats and by liver tissue to 7 of 17 (41%) random-bred Long Evans strain rats.

Peculiarities of Blood Coagulation in Patients with Cancer. JEROME M. WALDRON, M.D., B. BEIDLEMAN, M.D., AND G. G. DUNCAN, M.D., (Division of Med., Penna. Hosp., and Dept. Physiol., Jefferson Med. Coll.). Previous studies have shown that the oral ingestion of various fats, including cream from cow's milk, produces a sharp decrease in whole blood coagulation time. This hypercoagulability is detectable within ½ hour after drinking the cream and in 1 hour a maximum decrease in coagulation time averaging 45% is attained. Dilution of the fasting sample of blood with 0.85% sodium chloride solution produces first a decrease in coagulation time and with further dilution a return of the coagulation time to or beyond the time for whole blood. This is due to the differential effect of dilution on the accelerators and inhibitors of coagulation. Dilution of blood obtained after feeding the cream produces only a prolonged coagulation time because the hypercoagulability is due partially to the absence of effective inhibitors of coagulation. When the fasting blood sample is diluted with 10% cream in physiological saline solution, the coagulation time is decreased even on extreme dilution due to the clot-accelerating properties of the cream.

These characteristic responses are obtained not only in normal healthy individuals but in all groups of diseases encountered in a general hospital with one exception. This exception is patients with cancer. The response of patients with cancer to the oral ingestion of cream varies from a prolongation of coagulation time, which has not

been observed with any other disease, to a general minimal response which on the average does not differ appreciably from the normal variations in blood coagulation time obtained by repeated venipuncture. Dilution of this blood with cream in physiological saline solution does not result in a normal curve. This altered response of patients with cancer to the *in vivo* and *in vitro* clot-accelerating properties of cream is independent of the type or site of cancer in the cases studied. There is no correlation with the state of nutrition, sedimentation rate, or total plasma protein.

Some Observations on the Role of Carbon Dioxide in Acute Oxygen Toxicity at 3½ Atmospheres' Inspired Oxygen Tension. R. H. KOUGH, M.D., C. J. LAMBERTSEN, M.D., M. W. STROUD, M.D., R. A. GOULD, M.D., and J. H. EWING, M.D. (Depts. of Pharmacol., Med., and Psychiatry, Univ. of Penna.). To evaluate the role of CO_2 in convulsions produced by 3½ atm. of inspired oxygen tension 3 types of experiments were performed in man. In 4 men carried to convulsions at 3½ atm. the CO_2 tension of a subcutaneous gas depot in the forearm was followed as it approached equilibrium with the tissue pCO_2. In 7 men the blood flow (CBF), vascular resistance (CVR), and oxygen consumption (CMR_{O_2}) of the brain were measured by the N_2O method of Kety and Schmidt before and during O_2 breathing at 3½ atm. In 4 men arterial and internal jugular blood gases were measured during the breathing of 2% CO_2 in O_2 at 3½ atm.

The results at 3½ atm. O_2 were these. 1) The highest pCO_2 in the subcutaneous bubble was 50 mm. Hg. 2) A mean increase of 3 mm. Hg in the internal jugular pCO_2 was accompanied by a 5 mm. Hg decrease in the arterial pCO_2. 3) CBF decreased 25%; CVR increased 50%; and CMR_{O_2} was unchanged so that the internal jugular pO_2 averaged only 75 mm. Hg.

The significant finding in 7 experiments on the 4 subjects who breathed 2% CO_2 in O_2 at 3½ atm. was a decrease in the cerebral A-V O_2 difference, resulting in a mean internal jugular pO_2 of 800 mm. Hg.

In man it appears that any elevation of tissue pCO_2 accompanying acute oxygen poisoning at 3½ atm. is adequately explained by loss of the dual function of hemoglobin and changes in blood flow and it is not sufficient *per se* to produce convulsions. One way that the breathing of CO_2 might conceivably hasten the onset of oxygen convulsions is by increasing the CBF so that a larger bulk of the brain is exposed to higher, and presumably more toxic, levels of O_2 tension.

The Effect of Vegetable Fat Ingestion on Human Serum Cholesterol. EUGENE A. HILDRETH, M.D., S. M. MELLINKOFF, M.D., G. W. BLAIR, M.D., and D. M. HILDRETH, B.S. (Dept. of Int. Med., Hosp. of the Univ. of Penna.). There is now ample evidence that in most individuals the serum cholesterol concentration will fall if the intake of fat is sufficiently restricted. Some investigators believe that the chief reason for this chemical alteration is that the low fat diet automatically restricts the ingestion of cholesterol. However, animal studies suggest that the dietary fat level *per se* is more important than is the dietary cholesterol level in changing the serum cholesterol concentration. Our experiments were designed to discover whether human serum cholesterol concentration can be elevated by a diet of normal fat intake but low in cholesterol. Since there is evidence that vegetable fats contain no cholesterol, we investigated the effects of vegetable fat on the serum cholesterol concentration.

Three of the authors served as subjects. Each subject was studied, first, on his customary diet, which was es-

sentially a normal fat, normal cholesterol diet; second, when both fat and cholesterol were restricted; and third, when in a diet containing vegetable fats, cholesterol was restricted but total fat was normal in amount. The serum cholesterol concentration was followed twice weekly. The weights of the subjects and the protein and caloric content of the diet were maintained at a constant level throughout the study.

In these three healthy young males a restricted diet low in fat produced a significant fall in the serum cholesterol concentration. In all 3 subjects the subsequent addition to this diet of vegetable fat containing no cholesterol was associated with a rise in the serum cholesterol concentration to the control levels. Therefore, in these subjects, the restriction of total fat and not of cholesterol alone was the decisive factor in lowering the serum cholesterol concentration.

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BOOK REVIEWS AND NOTICES

BOOK REVIEWS

PEPTIC ULCER. By A. C. IVY, Ph.D., M.D., D.Sc., LL.D., M. I. GROSSMAN, Ph.D., M.D., and WILLIAM H. BACHRACH, Ph.D., M.D. Pp. 1144; 137 ills. Phila.: Blakiston, 1950. Price, $14.00.

THIS book is an authoritative compilation of existing knowledge of ulcer disease with emphasis on the physiologic aspects of the problem. It is attractively bound and well printed. The style of writing makes for easy reading and the subject matter is well documented and efficiently indexed. A commendable feature is the incorporation of summaries at the end of each chapter and each of the four parts into which the book is divided.

The section dealing with pathogenesis, to which approximately half the volume is devoted, is a notable condensation and evaluation of a wealth of experimental and investigative work. Diagnosis and treatment are likewise comprehensively and fairly discussed. The presentation of the clinical phases of the subject, however, would perhaps have been still further enhanced if the authors had been able to lend it that element of personal touch and experience which they so well succeeded in imparting to their discussions of the more purely laboratory and experimental aspects.

Of interest is the selection of the term "Peptic Ulcer" as the title for a work which keynotes the multiplicity of factors which may contribute to the production and maintenance of ulcer in man. The authors acknowledge that valid objections may be raised to such a cause-implying name for the disease under consideration. Nevertheless, they consider it the most satisfactory term, principally because they feel that generically it appropriately refers to nonspecific ulcers located in those regions of the alimentary tract exposed to gastric juice.

"Peptic Ulcer" undoubtedly represents the product of long and tedious labor on the part of many persons. In the opinion of this Reviewer, it is an outstanding accomplishment which should serve for years to come as an inspiration as well as a fount of information for all physicians and investigators interested in this important and perplexing disease.

J. B.

THE NEUROLOGICAL EXAMINATION. By RUSSELL N. DEJONG, M.D., Prof. of Neurology, Univ. of Michigan. Pp. 1079; 368 ills. New York: P. B. Hoeber, 1950. Price, $15.00.

DR. DEJONG has produced an exceedingly comprehensive, clearly written and logically arranged textbook which deals authoritatively with all aspects of the clinical neurological examination. This book will be of particular value to the postgraduate student of clinical neurology, in whose field it may be accepted as an official text. It is also of value to the undergraduate student and to the practicing neurologist and internist as a source of reference.

The anatomy and physiology of the central and autonomic nervous systems are thoroughly covered, and alterations in the structure and function of these systems are related to the clinical manifestations of neurological disease. Laboratory aids to diagnosis are fully described and their indications and limitations clearly presented.

Disease entities are not described as such, but are used to illustrate the various aspects of neurological disorders. Space has been devoted to the evaluation of disorders of consciousness.

The book is well printed and well illustrated and can be recommended without reservation.

J.L.

PATHOLOGIC PHYSIOLOGY: MECHANISMS OF DISEASE. Edited by WILLIAM A. SODEMAN, M.D., Prof. of Prevention of Tropical and Semi-tropical Diseases, Tulane Univ. of Louisiana. Pp. 808; 146 ills. Phila.: W. B. Saunders, 1950. Price, $11.50.

TWENTY-FIVE Authors, mostly internists, have collaborated to produce this excellent volume. An attempt has been made, and with very real success, to provide an analysis of the disturbed mechanisms underlying many of the symptoms and signs commonly observed in medical practice. No attempt has been made to supplant standard texts on physiology or medicine, but the reader is presented with a book which goes a long way toward bridging the gap existing between the

exhaustive treatise on Physiology, written by the physiologist, and the various clinical books which present in didactic fashion the etiology, symptoms, signs and treatment of disease.

The book has nine main divisions: Circulatory System by Drs. Sodeman, J. T. Roberts, F. D. Johnston, E. Hull, and J. S. LaDue; Respiratory System by Drs. J. H. Seabury and H. L. Alexander; Digestive System, by Drs. W. L. Palmer, J. B. Kirsner, L. Schiff, F. J. Ingelfinger, and R. Elman; Blood and Spleen by Drs. W. B. Castle and C. A. Doan; Urinary Tract by Drs. A. C. Corcoran and I. H. Page; Endocrine Glands, Water Balance, Nutrition by Drs. E. C. Reifenstein, Jr., R. L. Pullen, R. F. Rushmer, and W. A. Sodeman; Locomotor System by Dr. R. H. Freyberg; Infectious Diseases, Allergy by Drs. G. T. Harrell, Jr., R. A. Cooke, and W. B. Sherman; and Physical, Toxic and Chemical Agents by Drs. W. Bean and J. H. Foulger. It is to be noted that neurologic problems, metabolic disturbances, and disorders of acid–base balance are considered only as they are related to the various disease groups. It is unfortunate that some symptoms which are extremely common in general practice, for example, headache, vertigo, and fatigue, are almost entirely omitted. It is to be hoped that future editions will place more emphasis on psycho-dynamic phenomena in their relationship to disturbed physiology. Such limitations in scope are relatively minor, however, and this volume is heartily recommended to medical students and general practitioners. T. D.

THE BURDEN OF DISEASES IN THE UNITED STATES. By ALFRED E. COHN, Member Emeritus, Rockefeller Inst. Med. Research, and CLAIRE LINGG, Secretary, Former Committee on Research, New York Heart Assn. Pp. 129; 38 charts and figs., 11 tables. New York: Oxford University Press, 1950. Price, $10.00.

THIS is a distinctly unusual book. It is written by two authors well qualified for the purpose in hand: to weld into as consistent a description as possible the various and scattered pieces of existing information on the burden of diseases upon Society. They do not discuss the financial aspects of this burden, but implicit in their presentation is the purpose to draw from mortality and morbidity rates the material upon which sound planning can be made at a time when there is so much confusion in the problems of medical care. The source material is vital statistics in which "a world of living fact is buried in arrid tables". The Authors give vivid meaning to the changes which have taken place in causes of death effecting age groups from infancy to old age. Having shown the pattern as a whole, they then discuss separately each of the important diseases or groups of diseases.

The volume is generously illustrated with 38 well-conceived figures and charts and an additional eleven tables. Five of these charts are so extensive that they are contained in the separate transparent slip-case in which the volume is enclosed. They are beautifully multicolored creations, but the fact that 5 feet of space are needed to spread out one chart detracts from their practical usefulness.

From this study there emerges a very clear picture of (1) the great decline in the rate of deaths from infectious disease in youth; (2) the great increase in the number of deaths in the much augmented population of older persons; (3) the relatively few deaths that now occur in middle age; (4) a number of phenomena concerning several specific ailments—tuberculosis, diabetes mellitus, traumatic accidents, and cancer.

J. H.

PHYSIOLOGY OF SHOCK. By CARL J. WIGGERS. Pp. 459; 56 figs. New York: Commonwealth Fund, 1950. Price, $5.00.

FOR many years the Author of this monograph and his associates have contributed to our knowledge of the important syndrome of shock. This book is based upon these researches, but it also brings together and critically analyzes the vast amount of experimental work that has been carried on throughout the world. The manifestations of shock, as observed in patients and reproduced in animals, have been outlined, and the states to which the term "shock" should be restricted have been defined. The methods used in experimental research—work designed to duplicate nature's experiment as closely as possible—are given in detail. Later chapters deal with the mechanism of peripheral circulatory failure, with the effect of shock upon the heart, upon the respiratory and oxidative functions, with the accompanying metabolic disturbances, the toxemic and neurogenic factors, and with the involvement of special organs (such as adrenals, liver, and kidneys).

The concluding chapter is a summary of the sequential reactions in the development of shock and a brief outline of the more urgent problems of basic and practical importance that as yet are unsolved. These few pages are not the least valuable of a valuable text, for here Dr. Wiggers, from his own rich experience, states what studies he thinks merit preference in the design of further work. His book is a welcome addition to medical literature. B. L.

NEW EDITIONS

A Textbook of Gynecology. By ARTHUR HALE CURTIS, M.D., and JOHN WILLIAM HUFFMAN, M.D., Northwestern Univ. 6th ed. Pp. 799; 466 ills., 37 in color. Phila.: W. B. Saunders, 1950. Price, $10.00.

THE sixth edition of "A Textbook of Gynecology" is a joint effort of Curtis and Huffman. It is divided into 54 chapters and is profusely and excellently illustrated with photographs of gross and microscopic pathologic specimens and drawings by Tom Jones.

The first 8 chapters are devoted to normal pelvic anatomy, embryology, and physiology, and a discussion of the endocrine glands and clinical endocrinology. Included in the latter section are brief summaries of the methods for assaying certain of the hormones and other clinical diagnostic aids which are important in the study of patients with suspected endocrine abnormalities.

The section on pelvic infections is, as in previous editions, one of the most interesting and comprehensive of all the disease groups discussed. The place of the newer antibiotic and chemotherapeutic agents in treatment is included.

The diagnosis and treatment of pelvic cancer are thoroughly and clearly presented but the authors continue to deemphasize the value of cervical biopsy. They suggest that the gross pathologic picture is almost always so typical that therapy may be started without confirmatory evidence. The sections on benign uterine neoplasms, ovarian tumors, extrauterine pregnancy, pelvic relaxations, and lesions of the vulva and cervix are likewise well presented. In each instance both methods of diagnosis and of treatment are comprehensively discussed. Short but practical sections on the large intestine and the urinary tract as they affect gynecologic lesions are valuable.

This text is an excellent reference because it contains so much material and because it is so well indexed. The fact that in most instances the indications and techniques for the operative management of disease processes are more carefully discussed than the medical methods for controlling less spectacular abnormalities, such as atrophic vaginitis or amenorrhea, makes this book somewhat less valuable than it could be for the student and the general practitioner. It can, however, be recommended as an excellent general textbook in gynecology. J.W.

Williams' Obstetrics. By NICHOLSON J. EASTMAN, Prof. of Obstetrics, Johns Hopkins Univ. 10th ed. Pp. 1200; 696 ills. New York: Appleton-Century-Crofts, 1950. Price, $12.50.

THE purpose of the author, "to set forth in a comprehensive manner the principles and the practice of modern obstetrics for both medical students and practitioners", has been achieved in the 10th edition of Williams' Obstetrics. The material is divided into 10 sections (43 chapters) covering normal anatomy and physiology, the management of normal and abnormal pregnancy, and the care of the newborn. It has been brought up to date not only by the addition of new material but by the deletion of outmoded or disproved ideas which had been included in previous editions merely for their historical interest. The major advances in the history of the development of obstetric practice, however, have been retained.

Among the important additions is a section on "Orientation" which includes, in addition to the definition and derivation of the term obstetrics, statistical information concerning the birth rate, maternal and fetal mortality and explanations for the progressive decrease in both, abortion and the relationship of obstetrics to other branches of medicine. Other sections, including those on toxemia, endocrinology, contracted pelvis, dystocia, infection, hemolytic disease of the newborn and postpartum hemorrhage, have been completely rewritten. The new concepts of placental physiology and of the physiology of the uterine contractions have been included and their practical aspects discussed. Many other sections have likewise been revised or rewritten and many new and excellent illustrations have been added.

The manner in which controversial issues are presented makes the entire book most impressive. All sides of such issues are discussed and the author in each instance then outlines clearly and concisely his method of therapy; this alone makes it an excellent text for students and general practitioners. The lucid presentation of the more theoretical aspects of obstetrics and the extensive bibliography of the pertinent literature, both recent and older, make it an important reference book for the more advanced practitioner.

J. W.

Adrenal Cortex. Edited by ELAINE P. RALLI, KONRAD BLOCH, and GREGORY PINCUS. Trans. of the 1st Conference, November 21-22, 1949. Pp. 189; 20 ills. New York: Josiah Macy, Jr. Foundation, 1950. Price, $2.00.

A verbatim account of an informal conference among 15 outstanding experts on the chemistry, physiology, metabolism, and clinical applications of adrenal corticosteroids and ACTH. This small volume brings together the most recent knowledge in a new and exceedingly important field, and is recommended without reservation to those interested in the application of fundamental knowledge to human disease. R.K.

The Practice of Medicine. By JONATHAN CAMPBELL MEAKINS, M.D., Formerly Prof. of Medicine, McGill Univ. 5th ed. Pp. 1558; 518 ills., 50 in color. St. Louis: C. V. Mosby, 1950. Price, $13.50.

IN this new edition, the good qualities of previous editions have been maintained. The chapter on psychiatry has been replaced by an amplified section on psychosomatic medicine. A new chapter is devoted to the sulfonamides and the earlier antibiotics. The chapter on the ductless glands has been rewritten. R.K.

Handbook of Physiology and Biochemistry. By R. J. S. MCDOWALL, M.D., Prof. of Physiology, Univ. of London. 40th (Centenary) ed. Pp. 767; 315 ills. Phila.: Blakiston, 1950. Price, $7.00.

THE 40th or centenary edition of this well established book is a valuable adjunct for the person for whom it is intended—the medical student. Clear and easily understood charts and diagrams which are liberally used make for a quick grasp of important subjects.

The treatment is one of general principles and the simultaneous discussion of physiology and biochemistry are very good. The style is readable.

R. K.

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